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– studies on environmental exposure, disease susceptibility and epidemiology

Eeva Feuth (née Sajanti)

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To my family

ABSTRACT

Eeva Feuth (née Sajanti)

Lyme borreliosis in Finland – studies on environmental exposure, disease susceptibility and epidemiology

University of Turku, Faculty of Medicine, Institute of Biomedicine, Department of Medical Microbiology and Immunology, Doctoral Programme in Clinical Research (DPCR)

Annales Universitatis Turkuensis, Medica – Odontologica, Turku, Finland 2017

Lyme borreliosis is the most common tick-borne infectious disease in Europe and North America. It is caused by the spirochetes belonging to the *Borrelia burgdorferi* sensu lato complex. The bacteria are transmitted to humans by the hard ticks of the genus *Ixodes,* of which *I. ricinus* and *I. persulcatus* are present in Europe, including Finland. The first manifestation in the early phase of the infection is a red skin rash called erythema migrans. The manifestations of the disseminated disease vary from neurological symptoms to arthritis, chronic skin lesion, and on rare occasions to cardiac conduction disorders. The annual number of Lyme borreliosis cases has increased significantly during past decades in many European countries, the US, and Canada. At the same time, the abundance of vector ticks has increased and the geographical distribution has expanded towards northern latitudes in many studied areas such as Sweden, Russia and Canada.

The wide focus of this study targeted to investigate the tick distribution and associated pathogens in Finland, the epidemiology and seroprevalence of Lyme borreliosis, and host-related factors that increase susceptibility to the disseminated infection. According to our results, both *I. ricinus* and *I. persulcatus* are widely abundant in Finland, and approximately 16.9% of the ticks are infected with *B. burgdorferi* sensu lato. The incidence of Lyme borreliosis has increased significantly during the past two decades, and we estimated the total number of cases at present to be 6 000−7 000 yearly. Seroprevalence in the general Finnish population is 3.9%, with a higher prevalence occurring in males. No associations between chronic diseases (such as pulmonary diseases, autoimmune diseases, or cancer) and *B. burgdorferi* seropositivity were found in our study. Instead, deficiency in the lectin pathway of the complement seems to increase the susceptibility to disseminated Lyme borreliosis. These findings indicate that LB is an increasing public health concern in Finland, reflecting the situation in northern Europe in general.

Keywords: Lyme borreliosis, *Borrelia burgdorferi*, *Ixodes*, ticks, epidemiology, seroprevalence, MBL, Finland

TIIVISTELMÄ

Eeva Feuth (née Sajanti)

Lymen borrelioosi Suomessa – tutkimuksia ympäristöaltistuksesta, tautialttiudesta ja epidemiologiasta

Turun yliopisto, Lääketieteellinen tiedekunta, Biolääketieteen laitos, Lääketieteellinen mikrobiologia ja immunologia, Turun kliininen tohtoriohjelma (TKT)

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Lymen borrelioosi on yleisin puutiaisvälitteinen infektiotauti Euroopassa ja Pohjois-Amerikassa. Sen aiheuttaa *Borrelia burgdorferi* sensu lato -ryhmään kuuluvat spirokeettabakteerit. Ihminen saa bakteerin kovakuorisen *Ixodes*-suvun puutiaisen pureman välityksellä. Näistä *Ixodes*-puutiaisista kaksi, *I. persulcatus* ja *I. ricinus*, esiintyy Euroopassa. Ensimmäinen ilmentymä varhaisvaiheen borrelioosissa on puutiaisen puremakohtaan ilmaantuva punoittava ihomuutos, erythema migrans. Borrelioosin levinneessä muodossa taudinkuvat vaihtelevat neurologisista oireista nivel (artriitti)- ja iho-oireisiin sekä harvemmin sydänoireisiin. Lymen borrelioosi -tapausten määrä on kasvanut merkittävästi viimeisen kahden vuosikymmenen aikana useissa Euroopan maissa, Yhdysvalloissa ja Kanadassa. Samaan aikaan puutiaiset ovat runsastuneet ja niiden esiintymisalue on laajentunut pohjoisemmille leveysasteille esimerkiksi Ruotsissa, Venäjällä ja Kanadassa.

Tässä tutkimuksessa tarkasteltiin Lymen borrelioosia puutiaisvälitteisenä infektiotautina laajasta näkökulmasta. Tutkimuksessa selvitettiin puutiaisten levinneisyyttä ja niiden kantamien bakteerien esiintyvyyttä Suomessa, Lymen borrelioosin epidemiologiaa ja seroprevalenssia väestössä sekä infektion saaneen ihmisen ominaisuuksia infektiolle altistavina riskitekijöinä. Tutkimuksen mukaan Suomessa esiintyy runsaana sekä *I. ricinus* – että *I. persulcatus* –puutiaisia. Näistä 16.9 % kantaa *B. burgdorferi* sensu lato -bakteeria. Lymen borrelioosin ilmaantuvuus on kasvanut merkittävästi viimeisen kahden vuosikymmenen aikana ja tapausten vuosittaisen kokonaismäärän arvioimme olevan maassamme jopa 6000−7000. Seroprevalenssi suomalaisessa väestössä on 3.9 %. Miehillä seroprevalenssi on hieman korkeampi naisiin verrattuna. Kroonisten sairauksien (esimerkiksi keuhkosairaus, autoimmuunisairaus tai syöpä) ja *B. burgdorferi* seropositiivisuuden välillä ei ollut yhteyttä, mutta varhaiseen immuunipuolustukseen kuuluvan komplementtijärjestelmän lektiinitien puutteellinen toiminta näyttäisi altistavan disseminoituneen Lymen borrelioosin kehittymiselle. Nämä tulokset osoittavat, että Lymen borrelioosi on kasvava terveydellinen huolenaihe Suomessa heijastellen tilannetta myös muualla Pohjois-Euroopassa.

Avainsanat: Lymen borrelioosi, *Borrelia burgdorferi*, *Ixodes*, puutiaiset, epidemiologia, seroprevalenssi, MBL, Suomi

CONTENTS

ABBREVIATIONS

ACA	acrodermatitis chronica atrophicans
ANCOVA	analysis of covariance
AP	alternative pathway
APC	antigen-presenting cell
aqua-T	aqua containing tween
Avohilmo	Register for Primary Health Care Visits
Bbsl	*Borrelia burgdorferi* sensu lato
BL	borrelial lymphocytoma
bp	base pair
BSA	bovine serum albumin
CI	confidence interval
CXCL13	C-X-C motif chemokine ligand 13
CNS	central nervous system
CSF	cerebrospinal fluid
CP	classical pathway
DC	dendritic cell
DNA	deoxyribonucleic acid
ddH_2O	double-distilled water
EIU	enzyme immunoassay unit
EM	erythema migrans
ELISA	enzyme-linked immunosorbent assay
EUCALB	European Union Concerted Action against Lyme Borreliosis
Eur-TBEV	European subtype of tick-borne encephalitis virus
FE-TBEV	Far Eastern subtype of tick-borne encephalitis virus
FMSC	Finnish Microarray and Sequencing Centre
GEE	generalized estimating equation
HD	hospital district
HGA	human granulocytic anaplasmosis
Hilmo	National Hospital Discharge Register
HLA	human histocompatibility leucocyte antigen
HRP	horseradish peroxidase
ICD-10	International Classification of Diseases, revision 10
IFA	immunofluorescence assay
Ig	immunoglobulin
IL	interleukin
ITS	intergenic spacer
LA	Lyme arthritis

LB	Lyme borreliosis
LI	Lyme index
LNB	Lyme neuroborreliosis
LP	lectin pathway
MAC	membrane attack complex
MASP	MBL-associated serine protease
MBL	mannose-binding lectin
NIDR	National Infectious Diseases Register
NIHW	National Institute for Health and Welfare
NK	natural killer
NSS	normal sheep serum
OD	optical density
OR	odds ratio
Osp	outer surface protein
PBS	phosphate-buffered saline
PBS-T	phosphate-buffered saline containing tween
PCR	polymerase chain reaction
PLS	Post-Lyme syndrome
RF	relapsing fever
RNA	ribonucleic acid
ROC	receiver operating characteristic
rPCR	real-time PCR
RT-PCR	reverse transcriptase PCR
Sib-TBEV	Siberian subtype of tick-borne encephalitis virus
SNP	single-nucleotide polymorphism
ST	sequence type
TBE	tick-borne encephalitis
TBEV	tick-borne encephalitis virus
TLR	Toll-like receptor
TSLPI	tick salivary lectin pathway inhibitor
US	United States
WCS	whole-cell sonicate

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles which are referred to throughout the text by their Roman numerals.

I. Laaksonen Maija*, Sajanti Eeva*, Sormunen Jani J., Penttinen Ritva, Hänninen Jari, Ruohomäki Kai, Sääksjärvi Ilari, Vesterinen Eero J., Vuorinen Ilppo, Hytönen Jukka, and Klemola Tero. Crowdsourcing-based nationwide tick collection reveals the distribution of *Ixodes ricinus* and *I. persulcatus* and associated pathogens in Finland. Emerg Microbes Infect. 2017 May 10; 6(5):e31.

II. Sajanti Eeva, Virtanen Mikko, Helve Otto, Kuusi Markku, Lyytikäinen Outi, Hytönen Jukka*, and Sane Jussi*. Lyme borreliosis in Finland, 1995-2014: A nationwide register-based study. Emerg Infect Dis. 2017 Aug; 23(8):1282-1288.

III. Van Beek Janko, Sajanti Eeva, Helve Otto, Ollgren Jukka, Virtanen Mikko, Rissanen Harri, Lyytikäinen Outi, Hytönen Jukka*, and Sane Jussi*. Population-based *Borrelia burgdorferi* sensu lato seroprevalence and associated risk factors in Finland. *Manuscript submitted.*

IV. Sajanti Eeva M., Gröndahl-Yli-Hannuksela Kirsi, Kauko Tommi, He Qiushui, and Hytönen Jukka. Lyme borreliosis and deficient mannose-binding lectin pathway of complement. J Immunol. 2015 Jan 1; 194(1):358-363.

*These authors contributed equally to this article.

1 INTRODUCTION

Lyme borreliosis (LB), caused by the spirochetes of *Borrelia burgdorferi* sensu lato complex (hereafter referred to as "Bbsl"), is the most prevalent tick-borne infectious disease in the temperate zones of the northern hemisphere. Hard ticks of the genus *Ixodes* transmit the pathogen to humans during blood feeding. The first and most common manifestation of LB usually develops within two weeks in the skin at the inoculation site of Bbsl. This red skin rash characteristic of LB is called erythema migrans (EM). If the infection is left untreated and the immune system fails to eradicate the pathogen in the early phase, Bbsl might begin to disseminate via circulation or lymphatics to other tissues and organs. Various signs and symptoms of disseminated LB arise after a week to a few months and depend on the organ (e.g., central nervous system [CNS], skin, joint, heart) that Bbsl has affected. Antibiotics are used for treatment in all the manifestations, yet the late sequelae affect a proportion of patients despite the adequate therapy.

In Europe, around 85 000 LB cases are reported yearly and the incidence has increased in various countries during the past two decades (Lindgren *et al*. 2006, Smith and Takkinen 2006). The national incidence rates reported are mostly rough estimations due to a lack of uniform surveillance systems among countries. In addition to increased LB incidence in several European countries, vector ticks have become more abundant and they have spread towards northern latitudes and higher altitudes (Lindgren *et al.* 2000, Jore *et al.* 2011, Jaenson *et al.* 2012, Bugmyrin *et al.* 2013, Sormunen *et al.* 2016a). The same trend in increasing abundance and expanding range of vector ticks as well as an increasing trend in the incidence of tick-transmitted diseases have been noted on the other side of the Atlantic (Ogden *et al.* 2006, Dantas-Torres *et al.* 2012, Mead 2015). Meanwhile, the number of identified pathogenic microbes transmitted by ticks has increased. All these changes during past decades have warranted an increasing interest in tick-borne diseases among scientists, healthcare professionals working in the field of tick-borne diseases, and citizens.

The general aim of this study was to investigate the most important tick-borne disease, LB, with a wide focus. Study I focused on environmental exposure by examining the distribution of the vector ticks and associated pathogens in Finland. The study material, i.e., the tick collection covering the whole country and consisting of nearly 20 000 ticks, was gathered with the help of citizens based on so-called crowdsourcing. In studies II and III, the epidemiology of LB in Finland was thoroughly examined by using the data of three national healthcare registries and by examining the seroprevalence of LB in a subset of 2 000 Finnish adults. General

LB incidence rates, demographic characteristics, geographical distribution, seasonal variation, and different clinical manifestations of LB cases were examined. In study III, the host-related factors predisposing humans to disseminated LB were also investigated. In study IV, 350 serologically positive samples of LB patients and 350 serologically negative samples of non-LB controls were used to evaluate the MBL deficiency of the complement system as a predisposing factor to develop disseminated LB.

2 REVIEW OF THE LITERATURE

2.1 Ticks in nature

2.1.1 Ticks as pathogen vectors

Along with mosquitos, ticks are the most important arthropod vectors of pathogens to humans and companion animals worldwide (Jongejan and Uilenberg 2004). The number of different pathogen species transmitted by ticks is greater than any other group of blood-feeding arthropods (Pfäffle *et al.* 2013), and it has been increasing during past decades (Dantas-Torres *et al.* 2012). Meanwhile, the incidence of already well characterized tick-borne diseases, such as Lyme borreliosis (LB), has been increasing globally according to recent epidemiological reports (Mead 2015). In the United States (US), around 30 000 new LB cases are reported yearly to the Centers for Disease Control and Prevention but the actual number of cases is estimated to be tenfold. The incidence has increased approximately threefold during the past two decades and the geographical distribution of cases has expanded. (Mead 2015) In Europe, the estimated annual number of LB cases is 85 000, with the increasing incidence reported in several countries (Lindgren *et al.* 2006, Smith and Takkinen 2006).

With a total of around 900 species, ticks (*Acari: Ixodida*) are grouped into three families: the *Argasidae* (commonly known as "soft ticks", ~ 200 species), the *Ixodidae* ("hard ticks", over 700 species), and the *Nuttalliellidae* including only one species, *Nuttalliella Namaqua* (Sonenshine 1991, Anderson and Magnarelli 2008). The taxonomy of argasid ticks is not explicit, but a various number of genera from four to ten are listed depending on the source of information. Ixodid ticks consist of 13 recognized genera of which *Amblyomma, Dermacentor, Haemophysalis, Hyalomma, Rhipicephalus*, and *Ixodes* are worthy of mention in medical importance for humans. Altogether, at least 200 tick species, both argasid and ixodid ticks, have been reported to feed on humans occasionally, but around 30 are known to harbor and transmit pathogens to humans. (Anderson and Magnarelli 2008)

Most human cases of tick-borne diseases are related to hard ticks. Of these, the genus *Ixodes* transmits for example Bbsl, the causative bacterium of LB. *Ixodes* ticks are widely spread throughout the northern hemisphere, but there are differences in the geographical distribution of the species. Four species are mainly responsible for transmitting diseases to humans: *I. ricinus* (the castor bean tick) in Europe, south-western Asia, and northern Africa; *I. persulcatus* (the taiga tick) in

Asia and north-eastern Europe; *I. scapularis* in the eastern and upper midwestern US and south-eastern Canada; and *I. pacificus* in the western US (Lane *et al.* 1991, Stanek *et al.* 2012). The northernmost border of tick distribution in Europe and the overlapping distributional area of two human-infesting tick species (*I. ricinus* and *I. persulcatus*) both locate in Finland.

Due to the low mobility of ticks, the area for searching a host (questing) is relatively small in comparison with other arthropod vectors, e.g., mosquitos and flies. However, when blood feeding, ticks often stay attached to a host longer, many species even for a few days, which enhances the dispersal of pathogens to new environments (Estrada-Peña and de la Fuente 2014). Compared with other blood feeding arthropods, ticks also uptake a relatively large blood meal and direct more energy to survive rather long periods, even years, which reinforce their role as pathogen vectors. The small habitat, however, also makes them sensitive to changing climatic conditions, such as drought and low temperature.

Regarding the circulation of tick-borne pathogens in nature, there are a few important points in the relationship among ticks, pathogens, and hosts. The tick must acquire the pathogen (e.g., *B. burgdorferi*) from an infected host, maintain it through repeated moltings (transstadial transmission), and transmit it into a new host (Kahl *et al.* 2002). The host must be capable of serving as a reservoir for the pathogen, and subsequently, must be able to transmit the pathogen to the feeding tick. Some tick-borne pathogens (e.g., certain *Rickettsia* species) are transmitted from the engorged female to its eggs and thus, larvae are infective immediately after hatching (transovarial transmission). However, transovarial transmission has not been shown to occur in the case of Bbsl (Richter *et al.* 2012). Tick-borne diseases can occur only in the overlapping environments of vector-competent ticks, tick-transmitted pathogens, suitable reservoir hosts, and human exposure to ticks (Barbour and Fish 1993).

2.1.2 Tick biology and ecology

All ticks have four life stages: the embryonated egg, the larva, one (ixodid ticks) or more (argasid ticks) nymphal stages, and the adult tick. In most species, each active stage feeds once on the host and drops off to molt in the ground litter (3- or multi-host life cycle) (Estrada-Peña and de la Fuente 2014). From here on, the focus will be on *Ixodes* tick species, especially on *I. ricinus* and *I. persulcatus*, because these two hard ticks are capable of transmitting pathogens to humans and are present in Finland.

The feeding of *Ixodes* ticks begins relatively slowly and lasts from a few days (larvae and nymphs) up to two weeks (adults) (Estrada-Peña and de la Fuente 2014). The tick needs the blood meal for survival, for development from one life stage to the next, and for reproduction. The duration of the tick's life cycle is at least one year, but for species inhabiting cold climates, only the development from one life stage to the next takes approximately one year (Estrada-Peña and de la Fuente 2014). Thus, the completion of the whole metamorphosis from egg to adult tick may take up to three or four years (range: 1−6 years) (Randolph 2014).

Each active stage of the tick may prefer feeding on different groups of hosts. Immature stages (larvae and nymphs) commonly feed on small animals such as birds and rodents, whereas adults feed on medium-to-large-sized mammals, such as deer, livestock, and humans as incidental hosts (Tälleklint and Jaenson 1994, Estrada-Peña and de la Fuente 2014). Most ticks use relatively few hosts, yet there are species like *I. ricinus* and *I. persulcatus* that are known to feed on more than 200 hosts (Anderson 1989). This nature of general feeding may be important in bringing pathogens into new habitats and thus, exposing humans to novel bacterial species (Schotthoefer and Frost 2015).

Some ticks feed specifically on certain types of hosts (host-specific species), which makes their presence essential for the occurrence of ticks. Moreover, some of the hosts are necessary in maintaining and transmitting certain tick-borne pathogens in an area. For example, the enzootic cycle of a flavivirus causing tick-borne encephalitis (TBE) in humans involves certain small forest animals as reservoirs (Estrada-Peña and de la Fuente 2014). In the absence of these hosts, the pathogen will not circulate, although the tick may use other hosts for feeding. In addition to the density and composition of the reservoir host population, geographical barriers preventing the movements of host animals and thus restricting the distribution of ticks might be a limiting factor both for the tick and the pathogen occurrence (Estrada-Peña and de la Fuente 2014).

Apart from the above-mentioned host-related factors, there are also several environmental factors affecting the abundance and seasonal activity of ticks. The temperature, together with the photoperiod (hours of light in a day), regulates the development rates especially in cold climates, while reduced water availability (<80% relative humidity in vegetation) might increase the mortality of questing and molting ticks in dry regions. Long winters or unusually cold temperatures without a protective snow cover may reduce the survival of ticks that overwinter on the ground. (Estrada-Peña and de la Fuente 2014)

2.1.3 General morphology of I. ricinus and I. persulcatus

Ticks are small arachnids with eight legs typical to adult stages of this class. Larvae have only six legs but the fourth leg pair appears when they molt into nymphs. Unfed adults of *Ixodes* ticks usually range from 2−4 mm in size, but when blood-engorged, females may reach a length of over 10 mm. Nymphs are typically 1−2 mm and larvae even smaller, only around one-tenth of a millimeter. A hard dorsal shield, the scutum, covers the entire body of a male tick, but only the anterior part of females and nymphs. *Ixodes* ticks cannot be characterized into species by the naked eye. Under the microscope, however, differences can be seen: e.g., the length of the dorsal hair (long hair with *I. ricinus*, short with *I. persulcatus*), the lateral groove of the body (clearly visible in *I. persulcatus*, not visible in *I. ricinus*), and the genital aperture (directed downwards in *I. ricinus*, straight or wavy in *I. persulcatus*) (Hillyard 1996). The general features of both genders of *I. ricinus* and *I. persulcatus* are seen in Figure 1, but more detailed morphological characteristics are not discernible.

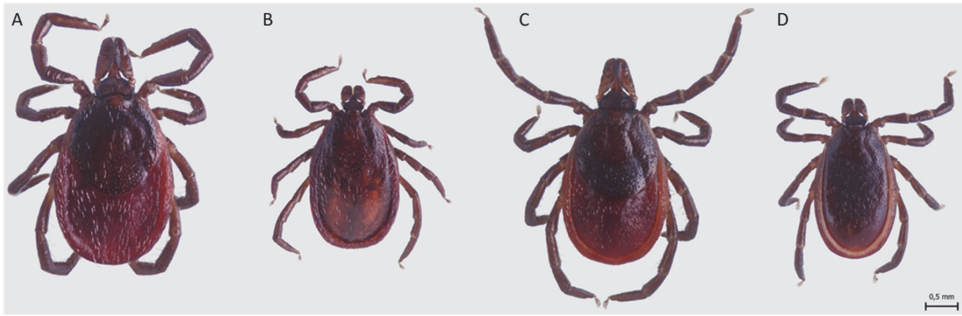


Figure 1. A) *I. ricinus*, female; B) *I. ricinus*, male; C) *I. persulcatus*, female; D) *I. persulcatus*, male. Photos: Maija Laaksonen, Department of Biology, University of Turku.

2.1.4 Seasonality of tick activity

Ixodes nymphs and adult ticks become active fairly concurrently in the early spring when temperatures exceed 7°C (Sonenshine 1991, Gray *et al.* 2009). In northern Europe, including Finland, both the unimodal (one activity peak per year) and bi-modal (two activity peaks per year) activity patterns of *I. ricinus* have been reported (Mejlon and Jaenson 1993, Gray 2008, Sormunen *et al.* 2016a). The second activity peak of *I. ricinus* questing adults may be seen in the autumn by nymphs that molted into adults after successful feeding during one summer. Larval activity peaks somewhat later than that of nymphs and adults. The seasonal activity of *I.*

persulcatus nymphs and adults has been reported to be unimodal, with a single activity peak occurring more or less at the same time as *I. ricinus* in the early spring (Tokarevich *et al.* 2011). *I. ricinus* ticks are active throughout the summer to the autumn, whereas the questing period of *I. persulcatus* is shorter (Gray 2008, Tokarevich *et al*. 2011).

2.1.5 Pathogen diversity and prevalence in ticks in Europe

LB, caused by the spirochetes of Bbsl, is the most important tick-borne disease in Europe (Stanek *et al.* 2012). The principal vector is *I. ricinus*, but in north-eastern Europe, *I. persulcatus* also transmit the bacteria. According to a meta-analysis of 155 European studies between 1984 and 2003, the overall mean prevalence of Bbsl in *I. ricinus* ticks was 13.7% (range: 0−75%), with the infection rate higher in adult ticks than in nymphs (18.6% vs. 10.1%) (Rauter and Hartung 2005). A significantly increasing trend in prevalence was seen in adult ticks when moving from west to east, and the highest infection rates were detected in central Europe (Austria, Czech Republic, Southern Germany, Switzerland, Slovakia, and Slovenia), although the overall prevalence of Bbsl in ticks varied greatly even within the countries. Much less research has focused on *I. persulcatus* ticks in Europe due to the limited geographical distribution of this species. However, in Estonia in 2013, where the distributional areas of both tick species overlap, the Bbsl prevalence of *I. persulcatus* (16.3%) was double that of *I. ricinus* (Geller *et al.* 2013). The updated data of the Bbsl prevalence in *I. ricinus* and *I. persulcatus* ticks in Europe is collected in Table 1.

The relapsing fever (RF) group of borrelia causing tick-borne relapsing fever form the other major group of the borrelia species. Bacteria are transmitted by soft ticks apart from one species, *Borrelia miyamotoi*, currently the only known RF borrelia transmitted by *Ixodes* ticks. After its discovery in *I. persulcatus* in Japan in 1995 (Fukunaga *et al.* 1995), the bacterium gained little attention until the first human infections were reported in Russia in 2011 (Platonov *et al.* 2011). Since then, *B. miyamotoi* has been detected with the prevalence of 0.5−4% in all *Ixodes* species capable of transmitting LB borrelia in several European countries, North America, and Russia (Wagemakers *et al.* 2015).

In addition to borrelia, ticks also transmit other important pathogens, of which the tick-borne encephalitis virus (TBEV) with thousands of cases annually in Europe, and the increasing incidence in many European countries including Finland, is of high medical interest (Lindquist and Vapalahti 2008, Tonteri *et al.* 2015). In Europe, the three known subtypes of the virus, European (Eur-TBEV), Siberian (Sib-

Table 1. *B. burgdorferi* sensu lato (Bbsl) prevalence in *Ixodes* ticks in some European countries. The prevalences collected in this table are only suggestive. The studies cover varying parts of the country, and the methods used to detect Bbsl prevalence vary from PCR to dark-field microscopy and immunofluorescence assay. Furthermore, there is great heterogeneity in prevalences among nymphs and adult ticks. Those studies selected for this table were the most recent found on PubMed, and concerning tick Bbsl prevalences in the named countries.

Species	Country	Prevalence (%)	No. of ticks			Year	Ref.
			Total	Adults	Nymphs		
I. ricinus	Austria	23.2	422	211	211	1989–2002	Hubalek 2003
	Belgium	23	489	45	444	1996	Misonne 1998
	Bulgaria	32.7	202	112	90	2000	Christova 2001
	Croatia	45	124	90	34	1995	Rijpkema 1996
	Czech Republic	17.3	526	214	526	2007	Kybikova 2017
	Denmark	15.0	661	-	-	2011	Stensvold 2015
	Estonia	8.2[a]	2 293	1 304	989	2006–2009	Geller 2013
	Finland (southwestern)	18.9	1 077	98	979	2013–2014	Sormunen 2016
	France	20.6	558	198	360	2008	Reis 2011
	Germany	20.7	2 110	673	1 437	2012	Mehlhorn 2016
	Hungary	40.8	240	240	0	2013	Hornok 2014
	Ireland	2.6–18.4	100–2 587	-	-	1990–1998	Rauter & Hartung 2005
	Italy (northern)	10.4–27.5	115; 292	-	-	2006; 2010	Corrain 2012, Aureli 2013
	Latvia	22.6[b]	517	-	-	1998–2001	Ranka 2004
	The Netherlands	13.2	6 025	-	-	2000–2011	Coipan 2013
	Norway (northern)	29.0	466	147	319	2011	Hvidsten 2015
	(southern)	22.1–31.3	1 579	449	1 130	2007	Kjelland 2010
	Poland	4.0–21.6	114–1 710	-	-	1993–2001	Rauter & Hartung 2005
	Portugal	3.9	2 095	1 087	1 008	2016	Nunes 2016
	Romania	25.8	534	77	457	2013–2014	Raileanu 2017
	Slovakia	10.2	670	-	-	2008–2010	Pangrácová 2013
	Spain	1.6	878	33	845	2012–2014	Espi 2016
	Sweden	27.9	1 331	388	923	2008–2009	Wilhelmsson 2013
I. persulcatus	Estonia	16.3[a]	540	427	113	2006–2009	Geller 2013
	Latvia	27.9[b]	523	-	-	1998–2001	Ranka 2004

[a]The overall prevalence of Bbsl in ticks was 9.7%.
[b]The overall prevalence of Bbsl in ticks was 25.3%.

TBEV), and Far Eastern (FE-TBEV), can be transmitted by various hard tick species, but the two most relevant for virus transmission are *I. ricinus* and *I. persulcatus* (Süss 2003). Eur-TBEV is mainly transmitted by *I. ricinus* in discrete foci within central and north-eastern Europe, whereas Sib-TBEV and FE-TBEV subtypes are transmitted by *I. persulcatus*, found similarly clustered in a belt-like area extending from north-eastern Europe across Russia and all the way to northern Japan. The overall prevalence of TBEV in ticks varies greatly depending on the geographical location and time of the measurement, but prevalences from 0.1% to 5% have commonly been reported in TBE endemic areas in Europe (Süss *et al.* 2002, Bormane *et al.* 2004). In Finland, Eur-TBEV and Sib-TBEV are endemic, and the overall prevalence of around 1−2% in ticks has been reported in studied areas (Jääskeläinen *et al.* 2010, Jääskeläinen *et al.* 2016).

Anaplasma phagocytophilum (formerly known as a granulocytic *Ehrlichia* group), the causative agent of a human granulocytic anaplasmosis (HGA), can be transmitted by various hard ticks across the northern hemisphere, but in Europe, the most important vectors are *I. ricinus* and *I. persulcatus* (Woldehiwet 2010). The geographical range of *A. phagocytophilum* practically covers all the countries where these ticks are endemic (Stuen *et al.* 2013). In a recent study conducted in southwestern Finland, the overall prevalence of 3.5% was detected in *I. ricinus* ticks, while elsewhere in Europe, the reported prevalence in *Ixodes* ticks has ranged from <1% to ~20% (Henningsson *et al.* 2015, Sormunen *et al.* 2016c, Stuen *et al.* 2013).

Candidatus Neoehrlichia mikurensis is a close relative of *A. phagocytophilum*. The bacterium, isolated from wild rats (*Rattus norvegicus*) and *I. ovatus* ticks in Japan in 2004, was identified to form a new clade in the family of *Anaplasmataceae* by phylogenetic analyses, and named as *Candidatus* N. mikurensis (Kawahara *et al.* 2004). A few years earlier, a closely related pathogen was already identified in *I. ricinus* ticks in the Netherlands (Schouls *et al.* 1999). To date, the pathogen has been detected in *I. ricinus* ticks in several European countries, such as Estonia, Sweden, Norway, Denmark, Germany, France, Switzerland, Slovakia, Czech Republic, and Hungary, with the prevalence varying from 1% to over 20% (Silaghi *et al.* 2016). Interestingly, it seems that *I. persulcatus* is less likely to carry *Candidatus* N. mikurensis, although infected ticks have been found, for example in the Asian part of Russia, where *I. ricinus* is not present (Alekseev *et al.* 2001, Rar *et al.* 2010, Ivanova *et al.* 2017). In Finland, *Candidatus* N. mikurensis has not been detected so far.

Tick-borne rickettsioses are caused by intracellular bacteria belonging to the spotted fever group of the genus *Rickettsia* (family *Rickettsiaceae,* order Rickettsiales) (Parola *et al.* 2013). Most of the dozen or so human-infesting *Rickettsiae* species present in southern and central Europe are transmitted by hard ticks other than *I. ricinus* or *I. persulcatus* (Parola *et al.* 2013). However, *I. ricinus* is a vector of

Rickettsia monacensis and *R. helvetica,* two *Rickettsia* species previously detected in northern Europe (Nilsson *et al.* 1999a, Svendsen *et al.* 2009, Severinsson *et al.* 2010, Katargina *et al.* 2015, Quarsten *et al.* 2015), and very recently identified in *I. ricinus* ticks in south-western Finland with the overall prevalence of *Rickettsia* spp. 1.5% (Sormunen *et al.* 2016b). In Estonia, *Rickettsia* species were also detected in *I. persulcatus* ticks, but the prevalence in *I. persulcatus* was much smaller than in *I. ricinus* (1.7% vs. 6.7%, respectively) (Katargina *et al.* 2015).

Other pathogenic species detected in *Ixodes* ticks in Europe include *Bartonella* spp. (Schouls *et al.* 1999), *Francisella tularensis* (Milutinović *et al.* 2008), *Pasteurella* species (Stojek and Dutkiewicz 2004), *Candidatus* Midichloria mitochondrii (Sassera *et al.* 2006), *Coxiella burnetii* (Movila *et al.* 2006), and *Babesia* spp. (Schorn *et al.* 2011). However, although a pathogen is detected in a tick, it does not directly imply that the tick can transmit the pathogen to a human. For example, bartonellosis caused by *Bartonella henselae,* which is also detected in *I. ricinus* ticks in Europe, was stated in a review not to be tick-borne (Telford and Wormser 2010).

2.2 Lyme borreliosis (LB)

2.2.1 Etiologic agent

The bacterium causing LB was first isolated from *I. dammini* ticks (currently known as *I. scapularis*) collected on Long Island, New York in 1982 (Burgdorfer *et al.* 1982). It was named *Borrelia burgdorferi* after Willy Burgdorfer, the medical entomologist who discovered the bacterium.

Of 21 identified Bbsl species (phylum: *Spirochetes*), *B. afzelii*, *B. garinii*, and *B. burgdorferi* sensu stricto are predominantly responsible for LB; *B. spielmannii* and *B. bavariensis* cause the disease less frequently; and *B. bissettii*, *B. lusitaniae*, *B. valaisiana*, and *B. kurtenbachii* have only occasionally been reported in human infections (Rudenko *et al*. 2011, Cutler *et al.* 2017). All three of these pathogenic and the six potentially pathogenic *Borrelia* species circulate in Europe, where the most common species responsible for LB is *B. afzelii* (Rauter and Hartung 2005). *B. burgdorferi* s.s. was previously considered the only pathogenic borrelia species circulating in North America. Last year, a novel borrelia species named *Candidatus* B. mayonii was identified from the clinical specimens of LB patients in the US, and the bacterium was also found in *I. scapularis* ticks (Pritt *et al.* 2016). In addition, three more Bbsl species present in the US (*Candidatus* B. andersonii, *B. Americana,* and *B. kurtenbachii*) are potentially pathogenic for humans (Clark *et al.* 2013, Clark *et al.* 2014). Pathogenic and potentially pathogenic Bbsl species are listed in Table 2.

Table 2. Pathogenic and potentially pathogenic *B. burgdorferi* sensu lato species. Modified from Borchers *et al.* (2015).

Species	Name confirmed	Vector	Geographic distribution	Cultured or isolated from	PCR positive
B. burgdorferi sensu stricto	1984	*I. ricinus, I. pacificus, I. scapularis*	USA, Europe	EM, blood, synovial fluid, CSF, heart, ACA	CSF, blood, synovial fluid/tissue
B. afzelii	1994	*I. ricinus, I. persulcatus*	Europe, Asia	EM, rarely blood, synovial fluid/tissue, CSF, ACA, BL	CSF, blood, synovial fluid, heart
B. garinii	1992	*I. ricinus, I. persulcatus*	Europe, Asia	EM, rarely blood, synovial fluid/tissue, CSF, ACA, BL	CSF, blood, synovial fluid
B. spielmannii	2006	*I. ricinus*	Europe	EM	
B. bavariensis	2013	*I. ricinus, I. persulcatus*	Europe, Asia	EM	EM
B. bissettii	2016	*I. ricinus,I. pacificus, I. scapularis*	Europe, USA	EM, CSF, BL	serum, blood, heart
B. lusitaniae	1997	*I. ricinus*	Europe, North Africa	EM	
B. valaisiana	1997	*I. ricinus*	Europe, Asia		skin, blood, CSF
B. kurtenbachii	2014	*I. scapularis, I. ricinus*	Europe, USA	CSF, skin	
Candidatus B. mayonii	2016 proposed	*I. scapularis*	USA	blood	blood, synovial fluid
Candidatus B. andersonii	1995 proposed	*I. dentatus*	USA		blood, skin
B. americana	2010	*I. pacificus*	USA		blood, skin

Abbreviations: EM=erythema migrans; CSF=cerebrospinal fluid; ACA=acrodermatitits chronica atrophicans; BL= borrelial lymphocytoma.

Borrelia is a long (10−30 µm), thin (0.18−0.25 µm), coiled, and highly motile bacterium with 7−11 bundled periplasmic flagella inside the double-layered (inner and outer membrane) cell wall (Barbour and Hayes 1986). The flagella enable the bacterium to keep the spiral-shaped morphology and move through viscous tissues (Motaleb *et al.* 2000, Zhao *et al.* 2014). In contrast to other Gram-negative bacteria, borrelia does not contain lipopolysaccharides, but rather a high density of different lipoproteins anchored to the outer membrane (Takayama *et al.* 1987, Kelesidis 2014) . Lipoproteins are important for the pathogenesis of borrelia, for example by enabling the bacterium to evade the immune system and adhere to the host (Zhao *et al.* 2014). Also, the genome of borrelia differs from other bacteria: genes that are necessary for the natural cycle of the pathogen locate in linear and circular plasmids (the number varying from 11 to 21), whereas one linear chromosome of approximately 900 000 base pairs (bp) is not related to the virulence or the host-pathogen interaction (Fraser *et al.* 1997).

2.2.2 Enzootic cycle of B. burgdorferi sensu lato *(Bbsl)*

Only in the overlapping environments of ticks and suitable reservoir hosts, Bbsl are capable of circulating in nature. By blood feeding on infected vertebrate animals, ticks acquire pathogens that they retain through repeated moltings and subsequently transmit to a new host (Kurtenbach *et al.* 2006). Transovarial transmission (pathogen transmission from an engorged female tick to its eggs) has not been demonstrated for Bbsl, and thus larvae are thought to hatch uninfected (Richter *et al.* 2012). However, in a recent experimental study, the larvae of *I. ricinus* could transmit *B. afzelii* to rodents, warranting more research on pathogen transmission dynamics (van Duijvendijk *et al*. 2016). Futhermore, there are dissenting opinions about whether hard ticks other than *Ixodes* spp. have vector competence for Bbsl (Mather and Mather 1990, Lledó *et al.* 2014).

Several small rodents (e.g., mice, bank voles, and squirrels), insectivores, hares, passerine birds, and larger mammals (e.g., raccoon dogs) serve as reservoir hosts for borrelia in Europe (Lane *et al.* 1991, Tälleklint and Jaenson 1994, Pisanu *et al.* 2014, Wodecka *et al.* 2016). Although important in the tick life cycle, elk (*Alces alces*) and roe deer (*Capriolus capriolus*) do not seem to have reservoir competence for Bbsl (Tälleklint and Jaenson 1994, Rosen *et al.* 2012). Humans and their companion animals are considered incidental dead-end hosts without a role in the enzootic cycle of Bbsl (Kurtenbach *et al.* 2006). The enzootic cycle of Bbsl is presented in Figure 2.

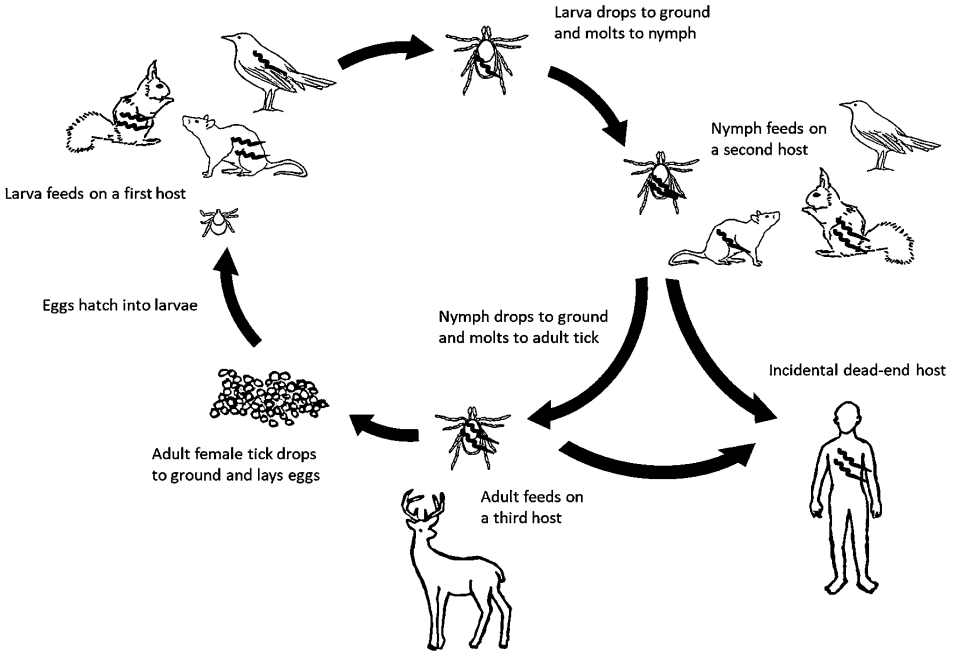


Figure 2. The enzootic cycle of *B.burgdorferi* sensu lato. *Ixodes* tick needs a blood meal to develop into the next life stage (from larva to nymph and from nymph to adult) and to reproduce. Bbsl is not passed from the adult tick to its eggs, so larvae hatch uninfected. While blood feeding on an infected host, each life stage of a tick might acquire the pathogen, which it retains through molting to the subsequent stage. Larvae usually feed on small mammals and birds, which are considered the primary reservoir hosts for Bbsl. Nymphs feed on a variety range of hosts and are thought to be the most important life stage of a tick in transmitting Bbsl to humans. As adult ticks, females attach to hosts for feeding, whereas males feed little and occupy hosts primarily for mating. Adult ticks usually feed on larger mammals, such as deer, that have no reservoir host competence for Bbsl. Although not important in the maintenance of Bbsl in nature, deer are important for the maintenance of the tick population because adult ticks commonly mate on them. Humans are incidental dead-end hosts without a role in the enzootic cycle of Bbsl. Modified from Radolf *et al.* (2012).

2.2.3 Factors influencing the risk of Bbsl infection

Obvious risk factors to obtain LB are time spent outdoors in tick-abundant areas during the questing season of ticks (Schotthoefer and Frost 2015). It has even been suggested that Bbsl infection of ticks might increase their host-finding efficiency (Faulde and Robbins 2008). The nymph is considered the most important life stage

in transmitting Bbsl to humans because the spring/early summer activity peak of nymphs occurs concurrently with human exposure to ticks due to the expected increase in outdoor activities (Rudenko *et al.* 2011, Borchers *et al*. 2015). Although higher Bbsl prevalence has been detected in adult ticks (Rauter and Hartung 2005), nymphs are far more abundant in tick populations (Sormunen *et al.* 2016a). The small size and the often undetected attachment in comparison with adult ticks further explains the importance of nymphs as pathogen vectors. Thus, the density of infected nymphs is the frequently used indicator to evaluate the human infection risk in an area (acarological risk) (Nicholson and Mather 1996, Stafford *et al.* 1998). Multiple prospective studies have also estimated the risk of developing LB after the tick bite (transmission risk), and in Europe it has varied from 3% to 12% (Maiwald *et al.* 1998, Nahimana *et al.* 2004, Fryland *et al.* 2011, Huegli *et al.* 2011, Wilhelmsson *et al.* 2016).

Molecular techniques, including deoxyribonucleic acid (DNA) sequencing, allow the investigation of pathogenicity not only between but also within the Bbsl genospecies (a term used to describe the different species of LB spirochetes) (Wang *et al.* 1999, Dykhuizen *et al.* 2008, Wormser *et al.* 2008, Strle *et al.* 2011, Hanincova *et al.* 2013, Jungnick *et al.* 2015, Coipan *et al.* 2016). Different sequence types (STs) of the same Bbsl species (e.g., *B. afzelii*) have been suggested to differ in their invasiveness, i.e., their capacity to cause disseminated LB in humans (Dykhuizen *et al.* 2008, Wormser *et al.* 2008, Strle *et al.* 2011, Hanincova *et al.* 2013, Jungnick *et al.* 2015, Coipan *et al.* 2016). The risk of developing manifest LB infection would thus also depend on the specific Bbsl genotype transmitted by the tick. Co-infection with other tick-borne pathogens, such as *A. phagocytophilum*, may also affect the susceptibility of disseminated LB. This is suggested to result from the capacity of *A. phagocytophilum* to infect neutrophils, and consequently to potentially impair the early defense against Bbsl (Thomas *et al.* 2001, Belongia 2002, Holden *et al.* 2005, Swanson *et al.* 2006).

Host related factors predisposing to Bbsl infection, other than those related to human behavior, are mostly unknown. However, certain human histocompatibility leucocyte antigen (HLA) -DR alleles are seen more often in patients suffering from antibiotic-refractory Lyme arthritis (LA), suggesting that certain HLA-DR haplotypes might influence the pathogenesis of LB (Steere *et al.* 2006, Kovalchuka *et al.* 2012).

2.2.4 Pathogenesis

During the blood feeding of an infected tick, Bbsl present in the tick's midgut undergo phenotypic changes by controlling the gene expression of certain outer surface proteins (Osps, such as OspA and OspC), which leads to the migration of spirochetes to the salivary glands of the tick (Radolf *et al.* 2012). From the salivary glands, spirochetes are deposited into the skin of a host, along with saliva containing anticoagulative and immunosuppressive bioactive agents (e.g., Salp15 and sialostatin L) (Ribeiro *et al.* 1987, Hovius *et al.* 2008). The process of spirochete migration from the tick's midgut to the host's skin is slow and requires tick attachment for more than 24 hours (Radolf *et al.* 2012).

If the infection is not resolved in the inoculation site, spirochetes begin to disseminate further to the skin and through blood to other tissues and organs. In the disseminated stage, both the innate and adaptive immune responses are important in controlling the infection and inflammation (Weis and Bockenstedt, 2010).

Bbsl do not produce toxins, but use host proteases (e.g. plasminogen, matrix metalloproteinases) from the immune cells in the degradation of the extracellular matrix when penetrating through tissues (Radolf *et al*. 2012). By expressing several outer surface proteins, Bbsl are able to adhere to tissue components through various receptors (Brissette and Gaultney 2014). These proteins mediate the adhesion to the vascular endothelium when the pathogen leaves the bloodstream to enter tissues, and are also important in helping the bacterium evade immune responses, e.g., complement- and antibody-mediated killing, and colonizing target tissues, e.g., synovial membrane of the joint. Host inflammatory responses are thought to be responsible for the tissue damage and hence, the pathology of LB. (Radolf *et al.* 2012)

2.2.5 Clinical manifestations

The spectrum of symptoms and clinical manifestations of LB are usually categorized into three stages: 1) the early, localized stage, 2) the early disseminated stage, and 3) the late disseminated stage. EM, a reddish/bluish-reddish skin rash, typically appears within 1−2 weeks (range: 1−129 days) at the tick bite site (Logar *et al.* 2004). During the following days to weeks, the patch expands radially, sometimes accompanied by local symptoms, such as pain or itching (Logar *et al.* 2004). "Flu-like" symptoms (fever, fatigue, headache, muscle and joint pain) and/or regional lymphadenopathy may occur (Nadelman *et al.* 1996, Logar *et al.* 2004, Stanek *et al.* 2011). Without treatment, EM eventually resolves, but the spirochetes may disseminate hematogenously or via lymphatics to other tissues and organs. In

20−30% of the cases in Europe, no EM appears (Berglund *et al*. 1995, Huppertz *et al.* 1999, Strle 1999). These patients are at particular risk of developing disseminated disease due to unnoticed and thus untreated early phase infection.

The early disseminated stage usually follows the localized infection after a week to few months and emerges as multiple EM lesions (in 5−40% of cases) (Berglund *et al.* 1995, Eriksson *et al.* 2013), a skin lesion called borrelial lymphocytoma (BL, ~5% of cases) (Berglund *et al.* 1995, Borchers *et al.* 2015), acute neurological symptoms (Lyme neuroborreliosis, LNB, ~20% of cases) (Stanek *et al.* 1987, Berglund *et al.* 1995, Lesnyak *et al.* 1998, Christova and Komitova 2004), or cardiac manifestations (≤1% of cases) (Berglund *et al.* 1995, Fish *et al.* 2008). Although each pathogenic Bbsl genospecies can cause any of the clinical manifestations of LB, the organotropism of different Bbsl species has been suggested in numerous studies (Borchers *et al*. 2015). LNB is strongly associated with *B. garinii* infection in European patients. *B. afzelii* causes predominantly different skin manifestations, whereas *B. burgdorferi* s.s. seems to prefer joints.

Multiple EM lesions, as well as BL, are seen more commonly in children than in adults (Berglund *et al.* 1995, Stanek *et al.* 1996). The highest rates of LNB occur in children and adults over 50 years (Hansen and Lebech 1992, Berglund *et al.* 1995, Huppertz *et al.* 1999, Henningsson *et al.* 2010), slightly more often in men than women (Borchers *et al.* 2015). Meningitis and uni- or bilateral facial palsy are typical neurological conditions in all age groups but in adults, meningitis presents together with painful radiculoneuritis (meningoradiculoneuritis i.e. Bannwarth syndrome) (Christen *et al.* 1993, Strle and Stanek 2009). Other neurological conditions, such as myelitis, encephalitis, and cerebral vasculitis, are rare (Stanek *et al.* 2011). Lyme carditis is a rare manifestation in European patients, affecting mainly people between 20 and 40 years and men more often than women (van der Linde 1991). Cardiac involvement predominantly manifests as atrioventricular conduction disorders occasionally requiring a temporary pacemaker, whereas myocarditis, pericarditis, endocarditis, pericardial effusion, ST-T wave changes, prolongation of the QT interval, and in rare cases, congestive heart failure may also occur (van der Linde 1991).

Late manifestations of LB may follow untreated early stage infection after months or years. Typical examples of late manifestations are LA and acrodermatitis chronica atrophicans (ACA). In Europe, LA is a fairly uncommon manifestation (around 5% of cases) possibly due to a higher occurrence of *B. afzelii* and *B. garinii* infections in comparison with *B. burgdorferi* s.s. (Oschmann *et al.* 1998, Rudenko *et al.* 2011). Brief, recurrent attacks of swelling and pain usually affect one or a few large, weight-bearing joints (typically the knee) (Borchers *et al.* 2015). Synovitis can be long-lasting and, although rarely, it may even cause destruction of the

joint resembling rheumatoid arthritis (Johnston *et al.* 1985, Steere *et al.* 1988). Fever and lymphadenopathy are relatively frequent co-findings (Borchers *et al.* 2015).

ACA, a late skin manifestation, is most commonly caused by *B. afzelii* and is thus almost exclusively seen in patients in Europe in <10% of LB cases (Borchers *et al.* 2015). It is characterized as a bluish-red skin lesion with swelling, and atrophy in the later stages, and often polyneuropathy on the extensor side of the extremity. ACA presents usually unilaterally, but may later become symmetrical. (Kindstrand *et al.* 1997)

Chronic LNB (symptom duration >6 months), seen very rarely in European patients, can arise as a progressive encephalomyelitis characterized by cognitive dysfunction, cerebellar ataxia, polyneuropathy, spastic bladder paresis, and para- and tetraparesis (Ackermann *et al.* 1988, Hansen and Lebech 1992, Oschmann *et al.* 1998). Occasional cases of ocular manifestations (uveitis, papillitis, keratitis, and episcleritis) have also been reported in any disseminated phase of the LB infection (Balcer *et al.* 1997, Zagórski *et al.* 2002).

2.2.6 Diagnosis

The diagnosis of LB is based on the clinical presentation in a patient with possible exposure to tick bites. According to European guidelines, typical EM can be diagnosed clinically and treated without any laboratory testing. In the case of atypical EM and in all the manifestations of disseminated LB, the laboratory-based confirmation of the infection is needed since the symptoms are not specific to LB and can result from various other medical conditions. The laboratory confirmation of LB is based mainly on the serology or detection of borrelial DNA in tissue and body fluid samples.

Serology has become a mainstay in the laboratory diagnostics of disseminated LB because the culture isolation of the pathogen from human samples is slow and rarely successful. The sensitivity of culture from EM skin biopsy is around 40−70%, while it is clearly less than 20% from other samples (e.g., cerebrospinal fluid [CSF], synovial fluid) (Borchers *et al.* 2015). According to the recommendations of the European Union Concerted Action on Lyme Borreliosis network (EUCALB) (Stanek *et al.* 2011), serological testing should be based on the two-step strategy where a sensitive enzyme linked immunosorbent assay (ELISA) is used as a screening test, and positive or equivocal result is further analyzed by a more specific immunoblot. However, the development of antibodies usually takes a couple of weeks after infection onset and therefore, serological testing likely yields a

negative result when performed in the early stage of the infection (Borchers *et al.* 2015). Thus, if LB is strongly suspected in a patient with a negative finding, the second serum sample should be tested 2−4 weeks later to demonstrate seroconversion. The LB diagnosis can also be based on the detection of Bbsl DNA by polymerase chain reaction (PCR) in tissue and body fluid samples (e.g., EM or ACA skin biopsy, synovial fluid), or, in the case of ACA, on the histology of the skin biopsy.

Despite being the mainstay of LB diagnostics, serological testing has certain limitations. When antibiotic therapy is started rapidly in the early phase of infection, seroconversion may never occur (Nadelman *et al.* 1996, Strle *et al.* 1996, Oksi *et al*. 2001, Glatz *et al.* 2006). In addition, Bbsl antibodies may remain elevated even years after adequately treated LB infection, thus challenging the serodiagnostics of the possible reinfection (Hammers-Berggren *et al.* 1994, Kalish *et al.* 2001b). Asymptomatic seroconversion occurs as well. In Europe, the background seropositivity in the general population ranges from 3% to 20%, but substantially higher seroprevalence rates have been reported in risk groups, such as forestry workers (Lakos *et al.* 2012, Borchers *et al.* 2015).

In the case of LNB, lymphocytic pleocytosis in CSF and the demonstration of intrathecal Bbsl-specific antibody production are required to confirm the diagnosis. However, CNS-derived antibodies may still be absent in the early phase of the infection (Blanc *et al.* 2007). In such a case, a CNS-derived lymphocyte chemoattractant, C-X-C motif chemokine ligand 13 (CXCL13), produced during neural inflammation, is a sensitive biomarker with a high specificity. Its concentration is elevated several days prior to intrathecal antibody production and declines rapidly in the course of antibiotic therapy. (Hytönen *et al.* 2014) Amplification of borrelial DNA from CSF by PCR lacks sensitivity but supports the diagnosis when positive (Nocton *et al.* 1996, Lebech 2002, Cerar *et al.* 2008).

2.2.7 Treatment and prevention

EUCALB has published treatment guidelines for different stages of LB based on available evidence of randomized clinical trials (Stanek *et al.* 2011). National treatment practices in Finland follow these recommendations (Hytönen *et al.* 2008). Antibiotic therapy is strongly recommended, as it prevents the development of other clinical manifestations of LB, hastens the clearance of infection, and prevents long-term sequelae in LNB (Johnston *et al.* 1985, Steere *et al.* 1983b, Kalish *et al.* 2001a).

Following the Finnish treatment guidelines and practices, EM is treated with oral amoxicillin for 2−3 weeks. Doxycycline is the drug of choice for patients who are allergic to penicillin, and azithromycin for those who cannot use doxycycline either (e.g., children <8 years, and pregnant and breastfeeding women). LNB and Lyme carditis are treated with intravenous ceftriaxone, whereas for ACA and LA oral antibiotics (amoxicillin, doxycycline) can also be considered. In general, the duration of antibiotic treatment is 2−4 weeks for the disseminated stages. (Hytönen *et al.* 2008) Recent studies have suggested oral doxycycline to be as effective as intravenous ceftriaxone in the treatment of LNB, but new treatment practices have not yet been adopted for use in Finland (Ljøstad *et al.* 2008, Kowalski *et al*. 2011, Bremell and Dotevall 2014). A clinical study comparing oral doxycycline as a primary treatment for acute LNB instead of intravenous ceftriaxone is ongoing in Finland (Prof. Jarmo Oksi, Turku University Hospital, personal communication).

Along with the antibiotic treatment, the symptoms of LB usually resolve gradually within weeks to months, but in a small group of patients, they may persist after adequate therapy. Residual neurological findings, e.g., remaining dysfunction of facial or other cranial nerves, radiculopathies, ataxia, and cognitive impairment, affect around 5−30% of LNB patients (Benke *et al.* 1995, Berglund *et al.* 2002, Ljøstad and Mygland 2010, Eikeland *et al.* 2012). In the case of LA, arthritis is prolonged without response to further antibiotic courses in around 10−15% of patients (antibiotic-refractory arthritis) (Huppertz *et al.* 1995, Bentas *et al.* 2000, Steere *et al*. 2006, Daikh *et al.* 2013). Ongoing inflammation of a joint seen 10−12 months after the initiation of therapy is thought to be caused by autoimmune responses (Borchers *et al.* 2015). Post-Lyme syndrome (PLS) is a term collectively used for unspecific symptoms, such as increased fatigue, emotional lability, myalgias and arthralgias, paresthesias, and sleeping, memory, and concentration disturbances, which persist for more than six months after therapy (Stanek *et al.* 2011). Prolonged courses of antibiotics are not recommended for long-term sequelae of LNB, antibiotic-refractory arthritis, nor for PLS because no evidence of symptomatic chronic Bbsl infection after recommended treatment exists (Oksi *et al*. 2007, Berende *et al.* 2010, Stanek *et al*. 2011, Borchers *et al.* 2015).

Preventive actions against LB are protective clothing when spending time in tick endemic areas, careful inspection of the skin, and quick removal of attached ticks. Until 2002, an OspA-based vaccine against LB (LYMErix, Smithkline Beecham) was available in the US market, but it was withdrawn due to concerns of adverse events related to vaccination and poor demand (Nigrovic and Thompson 2007). Tick bites without symptoms and signs suggestive of LB is not an indication for antibiotic treatment.

2.2.8 Epidemiology

Due to lack of uniform reporting practices and surveillance systems among countries, exact national incidence rates in the general population are mostly missing (Smith and Takkinen 2006). Epidemiological data is often derived from studies performed on populations at increased risk (e.g., forestry workers, hunters, or orienteers) or in endemic areas (Chmielewska-Badora *et al.* 2012, Vandenesch *et al*. 2014, Wilking and Stark 2014). However, available data suggest that LB cases are distributed throughout Europe with a decreasing incidence when moving from east to west in central Europe, northward in Scandinavia, and southward in Mediterranean countries (Hubálek 2009). The highest incidences are reported in central and northern Europe, and in Baltic countries (Table 3). In several countries and regions, the incidence of LB has increased throughout past decades, but has remained stable in others (Mead 2015).

EM is the most common clinical manifestation of LB in both Europe and North America (Asbrink *et al.* 1986, Stanek *et al.* 1987, Strle *et al.* 2002, Mehnert and Krause 2005, Mead 2015), but differences between the continents can be seen in the frequencies of clinical manifestations of disseminated disease. LNB is the most prevalent form of disseminated disease in Europe, whereas LA is more common in the US (Borchers *et al.* 2015). ACA and BL are exceedingly rare in the US. In European epidemiological studies, slightly more EM cases are reported in women than in men (Steere *et al.* 1983a, Stanek *et al.* 1987, Strle *et al.* 2002, Mehnert and Krause 2005, Bennet *et al.* 2007, Bacon *et al.* 2008). The bimodal age distribution, with disease peaking in school-age children and adults over 50 years, is similar in both sides of the Atlantic (Bacon *et al.* 2008, Vandenesch *et al.* 2014, Wilking and Stark 2014, Dessau *et al.* 2015, Nelson *et al.* 2015).

2.3 The immune response against Bbsl

2.3.1 Innate immunity

The innate immune system acts as the first line of defense against invading pathogens (Akira *et al.* 2006). It comprises physical barriers (e.g., protective skin and mucosa), a wide range of different immune cells (such as dendritic cells [DC], macrophages, neutrophils, and natural killer [NK] cells), the complement system, and inflammatory cytokines that coordinate the cell-mediated immune response.

When Bbsl are inoculated into the skin by a tick bite, DCs and sentinel macrophages are the first cells they encounter within the dermis (Benach *et al.* 1984).

Table 3. Reported estimates of LB incidence in some European countries. Data extracted from Smith and Takkinen 2006, Hubálek 2009, and Mead 2015.

Country	Year(s)	Incidence /100 000 population /y	Reference	Reported incidence change
Austria	2005	135	ST	
Belarus	2013	10.8	M	
Belgium	2005	16.0	ST, H	↑
Bulgaria	2005	13.0	ST, H	↑
Croatia	1993–2000	5.9	H	
Czech Republic	1989–2006	31.7	Aguero-Rosenfeld 2005	
Denmark	2012	1.2	M	↑
Estonia	2013	84.5	M	
Finland	2013	29.4[a]	M	↑
France	2009–2012	42.0	Vandenesch 2012	
Germany (eastern)	2002–2006	36.5	Fulop 2008	↑
Great Britain	2008–2009	1.7	M	↑
Hungary	2001–2005	12.8	H	
Iceland	2011	7.0	M	
Ireland	1995	0.6	H	
Italy	2001–2005	<0.1	H	
Latvia	2013	22.4	M	
Lithuania	2013	86.8	M	
Moldova	2003–2005	0.7	H	
The Netherlands	2005	103.0	Hofhuis 2006	↑
Norway	2012	5.1	M	↑
Poland	2012	22.8	M	↑
Portugal	1999–2004	0.1	H	
Russia	1999–2006	3.4–14.7[b]	H	
Slovakia	2008	19.2	Svihrova 2011	
Slovenia	2005	206.0	ST	↑
Sweden (southern)	1992	69.0	Berglund 1996	
Switzerland	1988–1998	25.1	H	
Ukraine	2012	3.6	M	

Abbreviations: y=year, ST=Smith and Takkinen 2006, M=Mead 2015, H=Hubálek 2009.
[a]The most recent incidence estimates are published in study II.
[b]depending on the region

These cells recognize the invading pathogens through pattern recognition receptors such as Toll-like receptors (TLR), which leads to the phagocytosis of the spirochetes (Medzhitov and Janeway 2000, Oosting *et al.* 2014). Activated macrophages produce proinflammatory cytokines (e.g., tumor necrosis factor α, interleukin [IL] 6, IL-8) that attract more neutrophils, T cells, macrophages, and DCs to the infection site (Porat *et al.* 1992, Petzke *et al.* 2009, Salazar *et al.* 2009, Oosting *et al.* 2011). This recruitment of immune cells eventually leads to the formation of EM (Duray 1989, Salazar *et al.* 2003). Macrophages and neutrophils are the central players in the innate immune response since they can attack the invading pathogens independently without the activation of the adaptive immunity (Connolly and Benach 2005). However, they are probably rather slow to move in comparison with motile Bbsl that might be able to escape them and avoid phagocytosis (Moriarty *et al.* 2008). After taking up spirochetes, DCs migrate to the lymph nodes, where they present the borrelial antigens to T cells and B cells and thereby activate these cells to participate in the defense (Section 2.3.2). DCs are therefore called antigen-presenting cells (APCs) and they act as a link between the innate and adaptive immunity. (Nussenzweig *et al.* 1980, Steinman 2012)

The complement system is another key component of innate immune defense that invading Bbsl instantly encounter when entering the skin. The complement system is discussed later (Sections 2.3.3 and 2.3.4).

2.3.2 Adaptive immunity

The adaptive immune system forms an antigen-specific response to invading Bbsl. It includes the antibody- and cell-mediated killing of bacteria, but its activation is slow and it depends on the stimulus of innate immune cells. T cells that are activated by APCs in lymph nodes, then enter the circulation and migrate to the infection site to assist macrophages in phagocytosis (helper T cells) and to directly kill infected cells (cytotoxic T cells) (Radolf *et al.* 2012). A number of helper T cells remain in lymph nodes and assist antigen-sensitized B cells to differentiate into plasma cells. Plasma cells secrete specific antibodies (immunoglobulins, Igs) that can kill Bbsl either by causing the lysis of the spirochetes via the complement system in the blood or by labelling the spirochetes (opsonization) for recognition and destruction by other components of the immune system (Connolly and Benach 2005). The T cell-mediated specific antibody production against Bbsl is essential for clearing the pathogen (Connolly and Benach 2005). Bbsl try to evade antibody-mediated killing by downregulating certain outer surface lipoproteins (such as OspC) and by using a mechanism called antigenic variation (Radolf *et al.* 2012).

2.3.3 The complement system – mannose-binding lectin (MBL) pathway

The complement system includes a complex collection of plasma proteins and cell membrane-associated receptors that are part of the innate immune system (Merle *et al.* 2015a). The activation of the complement can be initiated through three distinct pathways, which all eventually terminate in the formation of the membrane attack complex (MAC), a membrane-spanning pore that lyses the pathogen cell wall. These pathways are called the classical pathway (CP), the alternative pathway (AP), and the lectin pathway (LP). In addition to eliminating micro-organisms, the complement is also involved in waste disposal by facilitating the phagocytosis of apoptotic cells, and in the development of adaptive immune response by promoting the formation of specific antibodies and maintaining the immunological memory (Wagner and Frank 2010). Complement activation is strictly regulated to ensure that the efficient immune defense is achieved at the infection site, while healthy host tissue is protected against damage due to the inappropriate complement attack (Merle *et al.* 2015a). Both the complement deficiencies and the overactivation of the complement can be harmful for the host and are related to increased susceptibility to certain outcomes such as infectious diseases, autoimmunity (e.g. systemic lupus erythematosus), and cancer (Merle *et al.* 2015b). In this thesis, the focus will be on the LP of the complement and its role in the defense against Bbsl.

The LP can be activated by a family of proteins called collectins (e.g. mannose-binding lectin [MBL] and collectin-11) and ficolins (ficolin-1, -2 and -3) (Merle *et al.* 2015a). Because this thesis focuses on the MBL-initiated activation of LP, this pathway will hereafter be referred to as "the MBL pathway". MBL is a circulating and tissue-residing pattern recognition molecule that forms complexes with MBL-associated serine proteases (MASP-1, MASP-2, MASP-3, and sMAP) in a Ca-dependent manner (Thiel *et al.* 1997, Teillet *et al.* 2005, Wallis 2007). It is encoded by *MBL2* gene, located on chromosome 10 (Sastry *et al.* 1989, Taylor *et al.* 1989). Single-nucleotide polymorphisms (SNP) of this gene and its promoter region cause the impaired assembly of multimeric MBL molecules and the reduced capacity to form complexes with MASPs. This leads to the decreased concentration of biologically active MBL in blood and tissues, which in turn leads to the reduced efficacy of the MBL pathway-mediated protection against pathogens. (Sumiya *et al.* 1991, Lipscombe *et al.* 1992, Madsen *et al.* 1994, Garred *et al.* 2003) There are great differences in the frequencies of different MBL variant haplotypes between populations, for example Caucasians vs. Sub-Saharan Africans, but it is estimated that approximately one-fourth of the general population has deficient MBL pathway function due to homozygous or compound heterozygous variants (Heitzeneder *et al.* 2012).

MBL can bind to a variety of pathogens (bacteria, viruses, parasites, and fungi) by recognizing carbohydrate molecules (e.g., mannose and N-acetyl-glucosamine) on the pathogen cell wall (Ip *et al.* 2009, Endo *et al.* 2011). These sugars are rarely expressed on host cell surfaces but are commonly present on bacteria and viruses. The binding of MBL to the pathogen leads to the opsonophagocytosis and direct killing of the pathogen through the complement activation via the MBL pathway (Garred *et al.* 2009).

Impaired function of the MBL pathway occurs because of the low concentration of biologically active MBL in blood, which largely results from the MBL mutant genotypes. This state of impaired immune defense is commonly termed as 'MBL deficiency'. MBL deficiency has been reported to increase the susceptibility to various bacterial, viral, and parasitic infections (Eisen and Minchinton 2003, Ruskamp *et al.* 2006), such as respiratory tract infections, severe pneumococcal and meningococcal infections, sepsis, hepatitis B and C, human immunodeficiency virus infection, and malaria (Thomas *et al.* 1996, Garred *et al.* 1997, Hibberd *et al*. 1999, Roy *et al.* 2002, Cedzynski *et al.* 2004, Fidler *et al.* 2004, Thio *et al.* 2005, Eisen *et al.* 2006, Gordon *et al.* 2006, Das and Panda 2015). However, different definitions based on *MBL2* gene polymorphisms, MBL serum concentration, and MBL pathway functional activity measured by complement component (C4b) deposition assay have been used in disease association studies. There are some problems when the definition of MBL deficiency is based on the MBL variant genotype or MBL serum concentration alone. Although the homozygous MBL mutant variant leads to the total deficiency of MBL, heterozygous MBL variants may result in absent to a very high MBL serum level (over 10 000 ng/ml) (Minchinton *et al.* 2002). Thus, it is impossible to conclude the MBL serum level directly from the given *MBL2* genotype. Furthermore, age affects the MBL serum level so that the highest concentrations are measured in early childhood, around the age of 12 the concentration has decreased to the adults' level, and around 50 years of age, the serum level starts to decrease again (Aittoniemi *et al.* 1996, Sallenbach *et al.* 2011, Heitzeneder *et al.* 2012). In Finnish adults, the median MBL serum concentration of ~1100−4000 ng/ml has been reported in previous studies (Aittoniemi *et al.* 1996, Rantala *et al.* 2008, Gröndahl-Yli-Hannuksela *et al.* 2013).

It is not known which MBL serum concentration is sufficient to activate the MBL pathway, and different cut-off values from <50 to <1000 ng/ml have been used for deficient serum level (Heitzeneder *et al.* 2012). Neither MBL2 genotype nor the MBL serum concentration provide information on the functional activity of the MBL pathway. Although several studies have found associations between MBL deficiency and infectious diseases, inverse results have also been reported (Aittoniemi *et al.* 1998, Dahl *et al.* 2004). In intracellular infections, such as vis-

ceral leishmaniasis, tuberculosis, and leprae, MBL deficiency might even be beneficial because the opsonization and phagocytosis of the causative pathogens by macrophages are prevented (Garred *et al.* 1994, Hoal-Van Helden *et al*. 1999, Santos *et al.* 2001, Søborg *et al.* 2003).

2.3.4 Complement evasion of Bbsl with the focaus on the MBL pathway

When Bbsl are inoculated into the skin, the complement system becomes activated in order to eradicate spirochetes through phagocytosis and complement-mediated killing. All three complement activation pathways (CP, AP, and MBL pathway) are involved in Bbsl infection (de Taeye *et al.* 2013). The importance of CP and AP in the complement-mediated killing of Bbsl has been known for a long time, but the role of the MBL pathway was revealed rather recently (Kochi and Johnson 1988, van Dam *et al.* 1997, Schuijt *et al*. 2011).

Bbsl genospecies are classified into serum-sensitive (e.g., *B. garinii* serotypes 5 and 6) and serum-resistant isolates (e.g., *B. afzelii, B. spielmanii, B. bavariensis*) based on their ability to resist the bactericidal activity of human complement (de Taeye *et al.* 2013). The role of MBL pathway activation in Bbsl infection was demonstrated by Schuijt *et al*. in 2011, along with the discovery of a tick salivary protein (tick salivary lectin pathway inhibitor, TSLPI). TSLPI was found to inhibit the binding of MBL to its ligand on the surface of serum-sensitive *B. garinii* dose-dependently, and the spirochete was thus protected from the complement-mediated killing. (Schuijt *et al.* 2011)

In addition to above-mentioned TSLPI, Bbsl have multiple other tricks to use in the complement evasion. They recruit host complement regulators (factor H and factor H-like proteins) by expressing protective outer surface proteins (complement regulator acquiring surface proteins, CRASPs) and produce mimics of host membrane-bound complement regulators (such as CD59) in order to prevent the complement-mediated lysis (MAC formation). Furthermore, they utilize other tick salivary proteins than TSLPI (e.g., Salp15, Salp20) as well to avoid direct killing and phagocytosis induced by complement (Kraiczy *et al.* 2001, Pausa *et al.* 2003, Ramamoorthi *et al.* 2005, Pietikäinen *et al.* 2010, Meri *et al.* 2013, de Taeye *et al.* 2013). Figure 3 shows MBL pathway activation on the Bbsl surface leading to the formation of MAC.

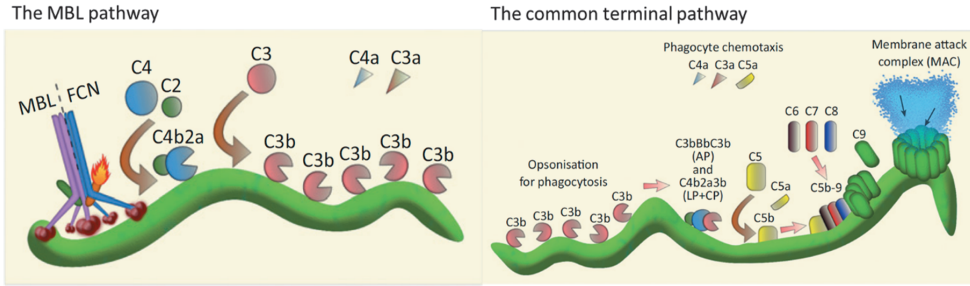


Figure 3. The activation of the MBL pathway of complement in *B. burgdorferi* sensu lato infection. MBL, in complex with MASPs or sMAP, recognizes carbohydrate structures on a pathogen cell wall, which leads to the cleavage of C2 and C4. This converges to produce C3 convertase consisting of C4b and C2a. The pathway proceeds step by step to eventually form MAC that lyses the pathogen cell wall. MBL=mannose-binding lectin; FCN=ficolin; C4=complement component 4; AP=alternative pathway; LP=lectin pathway; CP=classical pathway. Adapted from de Taeye *et al*. (2013) and reprinted with permission from Elsevier.

2.4 Other tick-transmitted infections

Little is known about possible tick-borne diseases other than LB and TBE in Finland. Research in the field of tick-borne diseases is currently very active, and new bacteria with possible pathogenicity to humans have recently been described. All of the tick-transmitted diseases presented below have not yet been encountered in Finnish patients, but the pathogens causing these infections are known to be present in ticks in Finland and patient cases have been published in neighboring countries.

2.4.1 Tick-borne encephalitis

The incidence of TBE has increased in Finland in past years (Tonteri *et al.* 2015). According to the National Infectious Disease Register (NIDR) (available in Finnish at: https://www.thl.fi/ttr/gen/rpt/tilastot.html), approximately 40−60 microbiologically confirmed cases (incidence ~1/100 000 population) are reported in Finland (including the Åland Islands) yearly. The risk areas for TBE are the Åland Islands and certain discrete foci by the coasts of the Gulf of Bothnia (for example, the archipelago of south-western Finland) and the Gulf of Finland (the archipelago of southern and south-eastern Finland) (Tonteri *et al.* 2015). TBE incidence is still the highest in the Åland Islands, although the incidence has significantly decreased

after the adoption of the TBEV vaccination in the national immunization program (Tonteri *et al.* 2015).

TBEV resides in the salivary glands of the questing tick, and the transmission of the virus from the saliva of the infected tick to the uninfected host takes only a few minutes. Therefore, quick removal of an attached tick does not prevent the TBEV infection. Clinical symptoms of TBEV infection follow around a week (range: 4−28 days) after the tick bite (Kaiser 1999, Haglund and Günther 2003, Lindquist and Vapalahti 2008). The disease course is usually biphasic: the first phase lasts a few days and is characterized by fever and general flu-like symptoms (malaise, fatigue, headache, muscle pain), and the second phase lasts a week to two months and is characterized by neurological signs and symptoms (e.g. headache, altered consciousness, delirium, spinal and cranial nerve paralysis, myelitis) arising from CNS infection (meningitis, encephalitis or myelitis). A symptom-free phase in between usually lasts around a week (range: 1−21 days). Subclinical infections occur frequently, and in 70−80% of people, the infection stops in the first phase without proceeding to CNS (Gustafson *et al.* 1992, Haglund and Günther 2003). Long-term neurological sequelae such as chronic headache, neuropsychiatric symptoms, and ataxia, are rather common. A mortality rate of 0−1.4% is reported in different follow-up studies in Europe (Mickiene *et al.* 2002, Haglund and Günther 2003, Wahlberg *et al.* 2006).

The diagnosis of TBE is based on the detection of anti-TBEV antibodies, especially IgM, in serum. In most cases, the serology is already positive in the acute phase of infection when CNS is affected. The viremia occurs even earlier during the first phase of infection, and on that time, TBEV could be detected by reverse-transcriptase PCR (RT-PCR) from blood. Instead, the sensitivity of TBEV ribonucleic acid (RNA) amplification from CSF in the acute second phase is poor. Intrathecal IgM and IgG antibody production is slow in TBE, and measurable concentrations are detected days after the anti-TBEV antibodies are found in blood. However, cross-reactivity in serology to other flaviviruses (e.g., dengue virus, West Nile virus, and Japan encephalitis virus) may hamper the diagnostics. Lymphocytic pleocytosis in CSF referring to viral CNS infection and abnormalities in electroencephalogram and/or cerebral magnetic resonance imaging may support the diagnosis although they are unspecific. (Lindquist and Vapalahti 2008)

The treatment is symptomatic without specific medications for TBE. In contrast to LB, an efficient vaccine consisting of inactivated viruses and providing protection against all the subtypes of TBEV (Eur-TBEV, Sib-TBEV, FE-TBEV) exists (Lindquist and Vapalahti 2008).

2.4.2 Other possible tick-transmitted infections in Finland

B. miyamotoi infection

B. miyamotoi, an RF borrelia, is one of the newly emerged pathogens. To date, different types of clinical pictures caused by *B. miyamotoi* have been described. The first report of *B. miyamotoi* infection was described in a case series of 46 patients in Russia in 2011 (Platonov *et al.* 2011). In these patients, the infection appeared as an unspecific acute febrile illness resembling influenza two weeks after the tick bite. Subsequently, similar reports of patients with *B. miyamotoi* infection have been published in the US and Japan (Krause *et al.* 2013, Krause *et al.* 2014, Sato *et al.* 2014). With some patients, the recurrence of fever episodes has been present (Platonov *et al.* 2011, Krause *et al.* 2016, Sudhindra *et al.* 2016). Three case reports of *B. miyamotoi* infection in patients with immunosuppressive treatment due to lymphoma have been described in the Netherlands, the US, and Germany (Gugliotta *et al.* 2013, Hovius *et al.* 2013, Boden *et al.* 2016). With two patients, *B. miyamotoi* infection manifested as chronic meningoencephalitis with progressive cognitive decline, memory deficits, and disturbed gait developing over months. With the German patient, the clinical picture resembled that of LNB with the acute onset of dizziness, headache, and vomiting. *B. miyamotoi* can be detected by PCR or by microscopy on blood and/or CSF in the acute phase of the infection (Platonov *et al*. 2011, Chowdri *et al.* 2013, Gugliotta *et al.* 2013, Hovius *et al.* 2013). Specific antibodies against the bacterium are found at least in the later course of the disease. The same antibiotics used in LB have been successfully used for *B. miyamotoi* infection. One case report from the US described a previously healthy man of 44 years with PCR- and serology-confirmed *B. miyamotoi* infection who recovered spontaneously without antibiotics (Sudhindra *et al.* 2016).

Human granulocytic anaplasmosis

A. phagocytophilum is an intracellular Gram-negative bacterium that infects neutrophils (Chen *et al.* 1994). The first confirmed patient case of HGA was reported in Slovenia in 1997 (Petrovec *et al.* 1997). Since then, altogether less than 100 confirmed patient cases have been described in several European countries, i.e., Sweden, Norway, the Netherlands, Spain, Croatia, and Poland (Blanco and Oteo 2002, Dugat *et al.* 2015). In most cases, HGA is a mild or moderate febrile illness with non-specific symptoms. Subclinical infections occur as well. However, in its' worst, the infection can be lethal (Dumler 2012).

Although HGA is infrequently detected in European patients, a seroprevalence of up to 28% has been reported (Strle 2004). In a very recent study conducted in Sweden in collaboration with the Åland Islands, the risk of developing clinical

disease (HGA) or seroconversion after a bite by *A. phagocytophilum* -infected tick was evaluated to be very low (Henningsson et al. 2015). Interestingly, the overall prevalence of *A. phagocytophilum* IgG antibodies was still 17% among the study participants although the prevalence of *A. phagocytophilum* in ticks that have bitten humans in the study was only 1.2%. Several explanations were offered by the researchers. For example, HGA might commonly be a mild or asymptomatic disease, only manifesting more severely in immunocompromised patients. *A. phagocytophilum* strains circulating in regions of high seroprevalence among humans might also be non-pathogenic, but may cause an immunological response when inoculated into the host's skin. Furthermore, the cut-off for seropositivity in immunofluorescent assay (IFA) used to detect IgG antibodies against *A. phagocytophilum* might have been set too low, or cross-reactivity with other bacteria caused false positives in the assay. The last explanation was also supported by Grankvist *et al.* who suggested that *Candidatus* N. mikurensis*,* which is a common bacterium in ticks in Sweden, might cause cross-reactive antibodies in IFA detecting anti-*A. phagocyptohilum* antibodies (Grankvist *et al.* 2015).

Neoehrlichiosis

The first identified patient infected by *Candidatus* N. mikurensis was a 77-year old Swedish man who had chronic B-cell lymphocytic leukemia, was using corticosteroids as a continuous medication, and had underwent splenectomy one month before the infection onset (Welinder-Olsson *et al.* 2010). His clinical disease was characterized by fever episodes, an erysipelas-like rash, and thromboembolic complications. *Candidatus* N. mikurensis was identified by sequencing the PCR product of the panbacterial 16S rRNA gene, which had been amplified from all blood culture bottles of the patient. In the same year (2010), another patient case of a 61-year-old man suffering from septicemia was described in Switzerland (Fehr *et al.* 2010). The Swiss patient wasa not immunocompromised due to disease or medication, but he had undergone coronary artery bypass graft surgery and mitral valve reconstruction six weeks prior. Furthermore, two German patients, the one immunocompromised due to medication and the other one previously healthy, obtained infections that manifested as systemic inflammation associated with thrombotic and hemorrhagic events (von Loewenich *et al.* 2010). Since then, more than a dozen cases in immunocompromised patients have been published in Europe (Silaghi *et al.* 2016). It has been suggested that immunocompetent hosts could carry *Candidatus* N. mikurensis long, even when the symptoms have already disappeared, and that the immunosuppressive treatment could reactivate the disease in these patients (Grankvist *et al*. 2015). In any case, *Candidatus* N. mikurensis has emerged only since 2010 as a potential pathogen and the knowledge of its ability to cause disease in humans is still limited.

Rickettsioses

In Europe, the first human infections caused by *R. monacensis* were reported in Spain (two patients) and in Italy (one patient) in 2005 (Jado *et al.* 2007, Madeddu *et al.* 2012). The diagnosis was obtained by molecular methods. The infection was characterized by fever and flu-like symptoms with all the patients, but an inoculation eschar was present only in one patient and generalized rash in two patients. Oral doxycycline was provided for treatment and all the patients recovered without sequelae.

Different kinds of patient cases related to *R. helvetica* infection have been published since 1999 (Parola *et al.* 2013). The clinical picture has varied from mild, self-limiting infection to septicemia and sudden death due to perimyocarditis (Nilsson *et al.* 1999b, Baumann *et al.* 2003, Fournier *et al.* 2000, Fournier *et al.* 2004, Nilsson 2009). In 2013, *R. helvetica* was suggested to be an etiologic agent of a proportion of patients with cranial neuritis in Sweden. In a study comprising 60 patients with facial palsy and 67 with sudden hearing loss, serological and molecular evidence of *R. helvetica* infection was found in around 8% and 12%, respectively (Nilsson *et al.* 2013). Apparently, the clinical picture of *R. helvetica* infection is still debated among scientists and all published patient cases have not been accepted by common consent to be caused by this pathogen (Parola *et al.* 2005).

Tularemia

Tularemia is a zoonosis caused by a Gram-negative intracellular bacterium, *Francisella tularensis*. The pathogen is widely distributed over the northern hemisphere, but different subspecies cause diseases in North America and Europe. *F. tularensis* subsp. *tularensis* (type A strain) which is considered the most virulent subspecies causes pulmonary infections almost exclusively in North America (Petersen *et al*. 2005). Its European counterpart, *F. tularensis* subsp. *holarctica* (type B strain), causes sporadic cases and outbreaks in many European countries. Especially in Finland and Sweden, the incidence of tularemia is relatively high in comparison with other parts of Europe, and outbreaks with hundreds of cases are reported recurrently (Rossow *et al.* 2015).

The symptoms and signs of tularemia result from different clinical forms of the disease, which largely depend on the portal of entry of bacteria. The ulceroglandular and glandular forms are the most common in Europe, and pathogen transmission occurs after animal contact or arthropod bite (Maurin and Gyuranecz 2016). In Finland, tularemia is typically a mosquito-borne infection (Rossow *et al.* 2014). After a short incubation of around 3−5 days, influenza-like symptoms fol-

low (Maurin and Gyuranecz 2016). If the disease is mild, it usually resolves without antibiotics. When more obvious clinical symptoms like inoculation ulcer, lymphadenopathy, or skin complications develop, the infection should be treated with fluoroquinolones or doxycycline. In the US, ticks are important in the transmission of tularemia whereas in Europe, their role as vectors is not considered so important. Several hard tick species, including *I. ricinus*, harbor *F. tularensis* but only a few tick-borne tularemia cases have been reported in Europe so far (Gurycová *et al.* 2010, Maurin *et al.* 2011, Bloch *et al.* 2013, Boone *et al.* 2015, Rojko *et al.* 2016).

Babesiosis

Babesiosis, caused by *Babesia microti*, is a relatively frequent tick-transmitted disease along the East Coast of the US, but in Europe, only sporadic cases of human babesiosis (caused mainly by *B. divergens,* another *Babesia* species), have been reported. *B. divergens* is a parasite of ruminants, and thus, most human infections have occurred in countries where the cattle industry is extensive, such as in Great Britain and France (Vannier and Krause 2009). In addition to *B. divergens*, *B. venatorum* and *B. microti* have also caused single *Babesia* infections in Europe (Herwaldt *et al.* 2003, Häselbarth *et al.* 2007, Moniuszko-Malinowska *et al.* 2016). Babesiosis often passes by without symptoms, but it may affect the kidneys or lungs in severe cases. After inoculation to a human by tick bite, *Babesia* invades the erythrocytes, eventually resulting in hemolysis. Symptoms like anemia, hemoglobinuria, acute renal tubular necrosis, and subsequent renal failure arise from hemolysis. Other complications of severe babesiosis include acute respiratory failure, congestive heart failure, disseminated intravascular coagulation, and splenic rupture. (Vannier and Krause 2009) Most of the cases reported in Europe (~30−40) have occurred in splenectomized patients, in whom the mortality rate has been as high as 42% (Hunfeld *et al.* 2008). In Finland, a fatal babesiosis case in a 53-year-old man without history of splenectomy was published in 2004 (Haapasalo *et al.* 2010). However, the man had a rudimentary spleen, his immune defense was generally decreased due to diabetes, and he had a history of heavy alcohol consumption. Moreover, he had a co-existing LB and he developed invasive aspergillosis during hospitalization. In the case of febrile hemolytic disease after a tick bite, babesiosis should be considered in the differential diagnostics especially in splenoctomized patients.

Some tick-borne diseases caused by selected pathogens that occur in Europe and could possibly occur in Finland are presented in Table 4.

Table 4. Lyme borreliosis, tick-borne encephalitis, and other selected tick-borne diseases in Europe.

Disease	Causative agent	Vector	Pathogen detected in Finnish ticks	Clinical picture, laboratory findings	Diagnosis	Patient cases reported in Europe	Patient cases reported in Finland
Bacterial							
LB	Bbsl	*I. pacificus, I. persulcatus, I. ricinus, I. scapularis*	yes	EM, LNB, LA, ACA, carditis, BL	Serology, PCR	Over 85 000 annually	Thousands annually
B. miyamotoi infection	*B. miyamotoi*	*I. ricinus, I. persulcatus, I. scapularis, I. pacificus*	yes	Fever, thrombocytopenia, leucopenia, elevated liver enzymes	PCR, microscopy, serology	A few cases	Not published
HGA	*A. phagocytophilum*	*I. ricinus, I. pacificus, I. scapularis*	yes	Fever, thrombocytopenia, leucopenia, elevated liver enzymes	Serology, PCR	Less than 100 confirmed cases	Not published
Neoehrlichiosis	*Candidatus* N. mikurensis	*I. ricinus,* possibly other hard tick species	no	Fever and systemic inflammation possibly with thromboembolic events	PCR	Less than 20 published cases	Not published
Rickettsioses[a]	*R. monacensis, R. helvetica*	*I. ricinus, I. persulcatus* (?)	yes	Fever, eschar, maculopapular rash, lymphadenopathy, different clinical pictures (*R. helvetica*)	PCR, serology	Single patient cases	Not published
Tularemia	*F. tularensis*	Various hard tick species, including *I. ricinus*	no	Fever, headache, vomiting, lymphadenopathy, inoculation ulcer	Serology, culture, PCR	A few cases[b]	Not published as tick-borne infections
Viral							
TBE	TBEV	*I. persulcatus, I. ricinus,* also other hard tick species	yes	Fever and flu-like symptoms followed by meningoencephalitis, myelitis	Serology	Annually	~40−60 confirmed cases annually
Parasitic							
Babesiosis	*Babesia divergens, B. venatorum, B. microti*	*I. ricinus, I. scapularis*	no	Fever, hemolytic anemia, petechiae, ecchymosis, leucopenia, thrombocytopenia, elevated liver enzymes	Microscopy, PCR, serology	~30−40 cases	One published patient case (Haapasalo 2010)

Abbreviations: LB=Lyme borreliosis; Bbsl=*Borrelia burgdorferi* sensu lato; EM=erythema migrans; LNB=Lyme neuroborreliosis; LA=Lyme arthritis; ACA=acrodermatitis chronica atrophicans; BL=borrelial lymphocytoma; PCR=polymerase chain reaction; HGA=human granulocytic anaplasmosis; TBE=tick-borne encephalitis; TBEV=tick-borne encephalitis virus.

[a]Only *R. monacensis* and *R. helvetica* are presented in this table. Other rickettsioses caused by different *Rickettsia* species are transmitted by other hard ticks and are distributed worldwide.

[b]Only the tick-borne tularemia cases are considered.

3 AIMS OF THE STUDY

I. To study the distribution of human-infesting *Ixodes* ticks and associated pathogens in Finland.
II. To evaluate the epidemiological situation of LB and to study changes that have taken place in LB incidence in Finland during the past two decades.
III. To examine *Borrelia burgdorferi* sensu lato seroprevalence in the Finnish adult population and to characterize host-related risk factors for LB.
IV. To study the influence of MBL deficiency of the complement system as a risk factor to develop disseminated LB.

4 MATERIALS AND METHODS

4.1 Ticks (I)

4.1.1 The Tickbank (I)

A total of 19 923 ticks forming the so-called 'Tickbank' were collected as part of the crowdsourcing-based, nationwide tick collection campaign in the year 2015. Via national newspapers, television, and internet, citizens were asked to send ticks (dead or alive) with information on the collection site and date and the species of the possible host via postal mail to the Department of Biology at the University of Turku. Ticks without adequate date information or those collected outside the campaign period (n=1 788) were excluded from the study. Thus, 18 135 ticks were analyzed in study I.

The species, life stage, and sex of the ticks were identified based on morphological characteristics under a microscope by trained and experienced biologists. The species are not distinguishable from each other by the naked eye, but can be discerned under the microscope based on certain characteristics in tick morphology. The species, life stage, and sex of 17 936 ticks could be determined morphologically. After the characterization, ticks were stored at -80ºC.

The geographical information of the ticks was conveyed to ETRS-TM35FIN coordinates with an accuracy of 100 m. Those ticks that lacked accurate collection site information (n=333) were excluded from the distribution analyses. Distribution maps were created using MapInfo Professional 12.0 software (Pitney Bowes Business Insight, Troy, NY, USA).

4.1.2 A subset of ticks for pathogen screening (I)

In order to investigate the prevalence of Bbsl, *B. miyamotoi*, and TBEV, a subset of 2 038 ticks, with an approximately equal number of *I. ricinus* and *I. persulcatus* were manually selected to represent the whole Tickbank by major collection areas, tick life stages, and sex distribution. However, due to our sampling method, a higher proportion of *I. ricinus* collected in May and June was analyzed for pathogens (84.1%) compared with their proportion in the whole Tickbank (60.5%). The species of 98 ticks (4.8%) that were included in this subset could not be reliably

identified under the microscope. For these, species-specific duplex real-time PCR (rPCR) assay was used.

The subsets of the ticks used in different analyses in study I are presented in Figure 4.

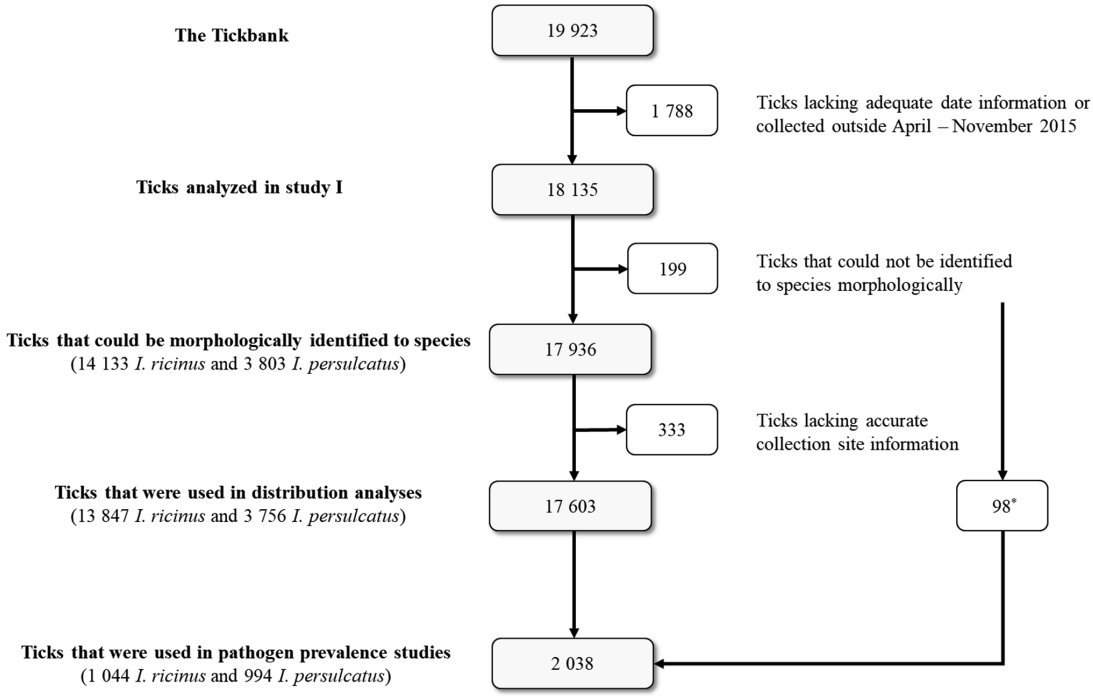


Figure 4. The flowchart shows the subsets of the ticks used in different analyses in study I.
*Of ticks that could not be identified morphologically by microscope, 57 were identified as *I. ricinus* and 41 as *I. persulcatus* by species-specific duplex rPCR.

4.2 Register data (II)

In Finland (current population 5.5 million), 20 geographically and administratively defined hospital districts (HDs) are responsible for the national health care system. The autonomous region of the Åland Islands is considered the 21st HD. The population in HDs ranges from 28 700 (the Åland Islands) to 1.6 million (Helsinki and Uusimaa). Sixteen HDs provide primary and secondary health care, and five also have tertiary care services. All HDs provide information to three national health care registers that were used to analyze the demographic characteristics, seasonality, clinical manifestations, and temporal and geographical distribution of LB in Finland. These registers were NIDR, National Hospital Discharge Register (Hilmo), and the Register for Primary Health Care Visits (Avohilmo) and they are maintained by the National Institute for Health and Welfare (NIHW). For this thesis, the epidemiological data of LB in Finland was updated to the year 2015 since the data of the year became available after the submission of the manuscript of study II.

4.2.1 National Infectious Disease Register (II)

The NIDR is maintained by NIHW based on the Finnish Communicable Diseases Act (1227/2016) and Communicable Diseases Decree (146/2017) (available in Finnish at: http://www.finlex.fi/fi/laki/smur/2016/20161227 [Accessed: 23rd July 2017]). Microbiological laboratories notify around seventy named pathogens and all pathogens that are isolated from blood or CSF to the NIDR.

This laboratory-based surveillance is also a routine in the case of LB. Since 1995, all microbiological laboratories performing LB diagnostics in Finland (n=8, including both public and private units) notify positive findings to the NIDR. Microbiological diagnosis is based either on serology or molecular methods. Each notification in NIDR includes the following information: specimen date and quality, each individual's unique national personal identity code, date of birth, sex, and place of residence. All microbiologically confirmed LB cases were extracted from the NIDR during 1995–2015. Within a three-month period, multiple notifications about the same person were combined as one case.

4.2.2 Avohilmo – Register for outpatient health care visits (II)

EM is the most common manifestation of LB and usually distinctive from other skin lesions when typical (Stanek and Strle 2009). When a typical EM is observed

after a possible tick exposure, it is diagnosed clinically without any laboratory testing and then treated with oral antibiotics by general practitioners in primary health care. Outpatient health care visits from the primary health care units (municipal health centers and health center wards) in the public sector have been registered to Avohilmo since 2011. Notifications include the patient's national identity code, age, sex, the place of health care service, the type and date of service (doctor consult or health care admission and discharge), investigations, treatment, and discharge diagnoses according to the International Classification of Diseases, revision 10 (ICD-10). Importantly, clinically diagnosed LB cases in primary health care (i.e., EM cases) are not reported to the NIDR since laboratory testing is missing.

In study II, LB cases were extracted from Avohilmo by the ICD-10 code "A69.2" referring to "Lyme borreliosis". Only the first discharge of each patient was included to avoid recurrent visits with the same diagnosis code being analyzed multiple times in the study. Avohilmo data were used to estimate the number of clinically diagnosed LB cases and to improve the estimate of the total number of LB cases in Finland during 2011–2015. For this, the microbiologically confirmed (NIDR) and clinically diagnosed (Avohilmo) LB cases were summed together and the following assumptions and corrections were used: 1) clinically diagnosed cases in primary health care (Avohilmo) do not significantly overlap with the microbiologically confirmed cases in NIDR, and 2) on average, 70% of LB diagnoses in Avohilmo are correctly coded by the general practitioners (based on registry-linked studies on an individual level, unpublished data). Furthermore, demographic characteristics, and temporal and geographical distribution of clinically diagnosed LB cases in Avohilmo were compared with the microbiologically confirmed cases in the NIDR.

4.2.3 Hilmo – Register for inpatient health care visits (II)

The Hilmo register is comparable to Avohilmo, but it contains notifications of inpatients from hospitals and outpatient clinics of hospitals from 1996 onwards. The proportions of different clinical manifestations of LB were determined using the Hilmo database from 1996 to 2014. LB cases with ICD-10 code "A69.2" (Lyme borreliosis), along with a more specific code referring to either LNB ("G01.9" meningitis, and/or "G63.0" polyneuropathy) or LA ("M01" arthritis in Lyme disease), were extracted from Hilmo. The diagnostic criteria for LNB include the clinical symptoms suggestive of CNS involvement with the laboratory confirmation consisting of CSF pleocytosis and the detection of intrathecally produced borrelia-specific antibodies and/or elevated CXCL13 concentrations in the CSF, or rarely, the amplification of Bbsl DNA from the CSF sample. LA is diagnosed in a patient

who has mono-/oligoarthritis accompanied by Bbsl-specific IgG antibodies in the serum or Bbsl DNA in a synovial fluid sample. These criteria are consistent with the case definitions recommended by EUCALB (Stanek *et al.* 2011).

4.3 Serum samples (III, IV)

4.3.1 Health 2011 serum samples (III)

The serum samples (n=2000) that were used to study the prevalence of antibodies against Bbsl in Finnish adults (III) were derived from blood donors from a national health examination survey (Health 2011 Survey), carried out by the NIHW in collaboration with several researchers and institutions in 2011. This survey was a follow-up study for the Health 2000 Survey conducted 11 years prior. Both surveys were aiming to collect information on the important national public health concerns and their determinants in the adult population with questionnaire and interview data, physical examination data, and laboratory measurements. The participants in Health 2011 Survey, without any specific exclusion criteria, were adults over 29 years of age living in the mainland Finland, thus representing the general adult population of the country. The Health 2011 Survey also includes linkage in Hilmo by using the national personal identity code. The data regarding diagnoses of specific chronic diseases (e.g., cancer, or pulmonary, skin, rheumatic, or autoimmune diseases) were obtained from Hilmo using their ICD-10 codes. The Health 2011 Survey is previously described elsewhere (Härkänen *et al.* 2016).

The median age in the subset of 2000 blood donors was 56 years (range 29−97 years) and 1 101 (55.1%) were females. The Bbsl seroprevalence based on this set of serum samples (III) was compared with LB incidence in Finland based on the health care register data (II). Furthermore, related risk factors and possible associations with other diseases and/or physical conditions were examined.

4.3.2 Patient serum samples (IV)

The serum samples that were used to study the possible association of deficient MBL pathway function with the susceptibility to LB (IV) were derived from the archived sera in the diagnostic laboratory at the Department of Medical Microbiology and Immunology at the University of Turku. The sera were originally collected as part of a routine clinical practice from individuals suspected to have LB. Anti-Bbsl IgM and IgG antibodies were tested from all samples by ELISA using

sonicated *B. burgdorferi* sensu stricto (strain: B31, ATCC 35210) whole-cell bacteria lysate as a coating antigen (Viljanen and Punnonen 1989). A flagella antigen-based ELISA (IDEIA Borrelia burgdorferi IgM/IgG; Oxoid, Cambridgeshire, UK) (n=245; 70% of samples) and/or a line IB using recombinant Bbsl antigens (*recom*-Line Borrelia IgM/IgG; Mikrogen, Neuried, Germany) (n=190; 54% of samples) were used as the following assays.

In the analysis, 350 serologically positive serum samples of LB-patients and 350 serologically negative serum samples of non-LB controls were used. The LB patient sample group included all the Bbsl positive samples tested in 2011 (350/~11 500 tested samples; 3.0%) and the number of samples in the non-LB control group (also from the same year 2011) was matched to this. In the LB-patient group, the median age was 62 years (range 2−89 years) and 169 (48%) patients were males. The non-LB control group included an equal number of samples of both genders (177 [51%] males) and of each age group (0−10, 11−20, 21−30 years, and so forth; the median age was 39 years, range 1−92 years) in order to obtain a baseline MBL concentration over age in the Finnish population. Thus, the LB patient group and the non-LB control group purposely did not match in regard to age.

4.4 Determination of the cut-off values for diminished MBL pathway function (IV)

The separate, existing data of 201 serum samples (median age 10 years, range 0−74 years; 100 [50%] males) with unknown LB status were used to determine MBL serum concentration cut-off values for diminished MBL pathway function, as described in study IV. From these samples, both the MBL pathway function as well as MBL serum concentration were measured simultaneously. The function of the MBL pathway was measured by a commercial enzyme-linked immunosorbent assay (Wieslab Total Complement System Screen Classical, MBL, Alternative Pathways; Euro-Diagnostica, Malmo, Sweden), which uses mannan as the activator for the MBL pathway and detects the formation of the MAC neoantigen (C5b-9) as an indication of complement activation (Seelen *et al.* 2005). The test was performed and the results were interpreted according to the manufacturer's instructions (diminished MBL pathway function <40%, deficient function <10%).

4.5 Tick DNA and RNA extraction (I)

DNA and RNA were extracted sequentially from the subset of 2 038 ticks that were selected for pathogen screening. Qiagen TissueLyser (Retsch, Haan, Germany) was used in the mechanical lysing of ticks, and DNA and RNA were extracted using NucleoSpin® RNA kits and RNA/DNA buffer sets (Macherey-Nagel, Düren, Germany) following the kit protocols. DNA extracts were stored at -20ºC and RNA extracts at -80ºC.

4.6 Real-time PCR assays (I)

4.6.1 Real-time PCR for tick species identification

Tick species, if unknown after morphological identification (n=98), was determined using a species-specific duplex rPCR assay as previously described (Sormunen *et al*. 2016b). Primers (IXO-I2-F4 and IXO-I2-R4) targeting a 94-bp fragment of *Ixodes* spp. internal transcribed spacer 2 gene (ITS2) were used to amplify genus-specific segments, and probes (Ipe-I2-P4 and Iri-I2-P4) were used to match the ITS2 region for either tick species (*I. persulcatus* or *I. ricinus*, respectively). The rPCR runs were performed at the Finnish Microarray and Sequencing Centre (FMSC; Turku, Finland) using QuantStudio 12K Flex Real-Time PCR System (Life Technologies Inc., Carlsbad, CA, USA). Positive (*I. ricinus* and *I. persulcatus* DNA samples confirmed by sequencing in an earlier study (Sormunen *et al*. 2016a)) and negative (double-distilled water, ddH_2O) controls were included in each run.

4.6.2 Real-time PCR and rRT-PCR for pathogen screening

Primers (Bbsl-ospA-F and Bbsl-ospA-R) and a probe (Bbsl-ospA-P) amplifying a 102-bp fragment of the *ospA* gene were used to detect Bbsl DNA (Ivacic *et al*. 2007). All runs included positive (*B. burgdorferi* sensu stricto strain B31 ATCC 35210) and negative (ddH_2O) controls.

B. miyamotoi was detected by rPCR with the primers (Bm-fla-F and Bm-fla-R) and probe (Bm-fla-P) targeting the *B. miyamotoi* flagellin gene (156 bp). DNA samples from *B. miyamotoi* confirmed by sequencing in earlier studies (Geller *et al.* 2012, Sormunen *et al.* 2016b) were used as positive controls; *B. burgdorferi* sensu stricto B31 (ATCC 35210) and ddH_2O were used as negative controls. The

rPCR runs targeting the *ospA* gene of Bbsl and the *flagellin* gene of *B. miyamotoi* were carried out by the LightCycler 480 II Real-Time PCR Instrument (Roche Diagnostics GmbH, Mannheim, Germany).

Pooled RNA samples were used for TBEV screening (10 samples per pool, 5 µl of each sample). The primers (F-TBEV1 and R-TBEV1) and probe (P-TBEV-WT) were used to amplify the 3'non-coding region of the TBEV genome by one-step rRT-PCR. The protocol was performed as previously described (Schwaiger and Cassinotti 2003, Tonteri *et al.* 2011). The rRT-PCR runs were carried out at FMSC using the QuantStudio 12 K Flex Real-Time PCR System (Life Technologies Inc.). Individual RNA samples were re-analyzed if a pooled sample was tested positive. Positive (TBEV-Sib and TBEV-Eur) (Jääskeläinen *et al.* 2006, Tonteri *et al.* 2013) and negative (ddH_2O) controls were included in each run.

4.7 Analyses of the serum samples (III, IV)

4.7.1 Testing algorithm for the Bbsl seroprevalence study (III)

All Health 2011 serum samples (n=2 000) were screened for IgG antibodies by in-house ELISA using *B. burgdorferi* sensu stricto (strain: B31, ATCC 35210) whole-cell sonicate (WCS) as a coating antigen. This assay provides the results as arbitrary enzyme immunoassay units (EIU). Screening-positive samples (WCS IgG result ≥20 EIU) (n=329) were further analyzed by the C6 Lyme ELISA Kit (Immunetics, Brussels, Belgium). Sera that yielded positive or equivocal results in C6 Lyme ELISA and sera that yielded negative test results in this assay but were clearly positive in WCS IgG in-house ELISA (WCS IgG result ≥40 EIU) were further tested with *recom*Bead IgG 2.0 (Mikrogen, Neuried, Germany) (n=164). The testing algorithm of study III is presented in Figure 5.

4.7.2 Borrelia whole-cell sonicate IgG in-house ELISA (III)

To prepare *B. burgdorferi* sensu stricto (strain: B31, ATCC 35210) WCS for IgG in-house ELISA, bacteria were cultured in the Barbour-Stoenner-Kelly II medium (Barbour 1984) at 33ºC and passaged once a week until it reached a concentration of 5−9x10^7 /ml. After culture, bacteria were washed four times with phosphate-buffered saline (PBS) containing 5 mM $MgCl_2$. Between the washings, centrifugation was performed at 8000 rpm for 30 min at 4−10°C by Sorvall Instruments RC-5C (GMI, Ramsey, MN, USA). Finally, bacteria were suspended in PBS and

sonicated 4 x 30s by Bandelin Sonoplus HD 2070 (BANDELIN electronic GmbH & co. KG, Berlin, Germany) with the maximum efficacy (70%). Sonicated bacteria were centrifuged 10 000 rpm for 30 min at 4−10°C to dissolve the lysed bacteria cell components from supernatant containing the antigen. Protein concentration was measured by the Pierce BCA Protein Assay Kit (Pierce Biotechnology, Thermo Fisher Scientific, Rockford, IL, USA) according to the manufacturer's instructions. Antigen was diluted in the 20 µg/ml concentration in PBS and stored at -70°C until use.

Microtiter plates (Thermo Fisher Scientific) were coated overnight at 37ºC with borrelia WCS. After incubation, the wells were washed twice with aqua containing 0.05% Tween20 (Merck, Darmstadt, Germany; aqua-T) and blocked 1h at 37ºC with PBS containing 1% normal sheep serum (1% NSS-PBS). After blocking, the wells were washed twice with aqua-T, and serum samples (diluted 1:100 in 1% NSS-PBS), a 0 EIU standard sample, and a 100 EIU standard sample (consisting of pooled positive samples) were added. Goat anti-human IgG γ-chain specific alkaline phosphate conjugate (Calbiochem, Merck, Darmstadt, Germany) used as the secondary antibody was diluted in 1:20 000 in 1% NSS-PBS. After adding the samples, standard, and controls, the rest of the protocol was performed automatically by the BEP III System (Siemens Healthcare GmbH, Erlangen, Germany). All the samples were tested in duplicate. Sera ≥20 EIU were assigned as positive (n=329). Since 1995, this assay has been in everyday use in routine LB diagnostics in the diagnostic laboratory of Microbiology and Genetics at the Turku University Hospital.

4.7.3 Immunetics C6 Lyme ELISA Kit (III)

Positive samples in WCS IgG in-house ELISA (n=329) were next tested manually by the C6 Lyme ELISA Kit (Immunetics) according to the manufacturer's instructions. In the indirect ELISA assay, the microwell plate is coated with conserved, highly immunogenic synthetic peptide (C6 peptide) derived from VlsE protein. The assay simultaneously detects IgM and IgG antibodies of the serum sample and provides the result as a Lyme Index value (LI), calculated by dividing the absorbance of the sample by the assay cut-off value. The absorbance was detected at 450 nm with a Multiskan EX spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). According to the interpretation criteria of the manufacturer, LI 0.91-1.09 is an equivocal result and LI ≥1.1 is a positive result. We analyzed all samples (n=146) that yielded a result LI ≥0.9 with *recom*Bead IgG 2.0 (Mikrogen). Furthermore, those samples (n=18) with LI <0.9 but with a clearly positive WCS IgG

result (WCS IgG result ≥40 EIU) were also tested with *recom*Bead IgG 2.0 (Mikrogen).

4.7.4 recomBead IgG 2.0 (III)

The *recom*Bead IgG 2.0 is based on semi-automated technology where microscopic, magnetic polystyrene beads (MagPlex beads) are coated with specific immunodominant antigens for Bbsl. The test uses 13 different antigens (p100, VlsE, p58, p39, OspA, OspC [*B. burgdorferi s.s.*, *B. afzelii*, *B. garinii]*, and p18 [*B. burgdorferi s.s.*, *B. afzelii*, *B. bavariensis*, *B. garinii*, and *B. spielmanii*]) to detect specific IgG antibodies from serum samples. The sample and reagent preparations were made manually according to the manufacturer's instructions and the analyses were performed automatically using the MAGPIX System with the Luminex® xPONENT software (Mikrogen). In the test, the fluorescence intensity is comparable to the number of bound antibodies on the surface of the beads. The test-specific interpretations of the bead measurements were made automatically with the Mikrogen *recom*Quant evaluation software. According to the interpretation of the software, the test result of 0−4 points is negative, 5−6 points is borderline, and ≥8 points is a positive result. In our analyses, 78 samples were negative, 65 were positive, and 21 received 5−6 points (IgG antibodies were mainly targeted to the VlsE and/or p100 antigens). These 21 samples (WCS IgG result ≥20 EIU, C6 Lyme ELISA LI ≥0.9, and seroreactivity in *recom*Bead IgG 2.0) were assigned positive.

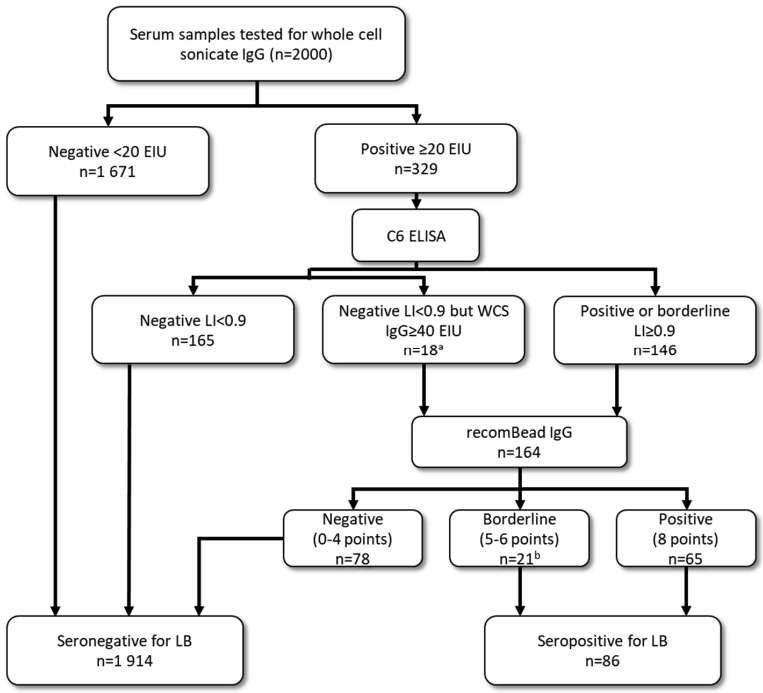


Figure 5. Testing algorithm for study III. [a]Only one of the 18 samples with LI <0.9 but with WCS IgG result ≥40 EIU was positive (16 points) and 17 received a negative result (≤4 points) in *recom*Bead IgG 2.0. [b]These 21 samples (WCS IgG result ≥20 EIU, C6 Lyme ELISA LI ≥0.9, and seroreactivity in recomBead IgG 2.0) were assigned as positive.

4.7.5 MBL ELISA methodology (IV)

MBL concentrations of the LB patient and non-LB control samples were measured using the double-antibody sandwich ELISA assay format. In short, microtiter plates were coated with mouse monoclonal anti-human MBL IgG1 antibodies (Statens Serum Institute [SSI], Copenhagen, Denmark) diluted in 50 mM carbonate-bicarbonate buffer (Sigma Aldrich, Steinheim, Germany) at the concentration of 8 µg/ml. PBS containing 1% bovine serum albumin (BSA; MP Biomedicals, Eschwege, Germany) was used to block nonspecific binding sites of the microtiter plate. The samples were diluted 1:100 in PBS containing 0.05% Tween20 (Merck; PBS-T) and the commercial standard serum containing 3 200 ng/ml oligomerized MBL (SSI) was used to create a standard curve. Negative (MBL-deficient serum [SSI]) and blank controls were included in each assay. Biotinylated mouse monoclonal anti-human MBL IgG1 (SSI) was diluted in 1:10 000 in PBS-T. Horseradish peroxidase (HRP) linked to streptavidin (Sigma-Aldrich, St. Louis, MO, USA) diluted in 1:8 000 in PBS-T was used to cause the detectable colour

change in the assay. The absorbances were detected at 450 nm with a Multiskan EX spectrophotometer (Thermo Fisher Scientific) and the optical densities (OD) were converted to serum concentrations (ng/ml) by using the created standard curve. The concentrations below the assay detection limit (set at 50 ng/ml) were given a value of 25 ng/ml as a hypothetical mean between 0 and 50 ng/ml. All samples were tested in duplicate. A schematic drawing of the sandwich ELISA is presented in Figure 6.

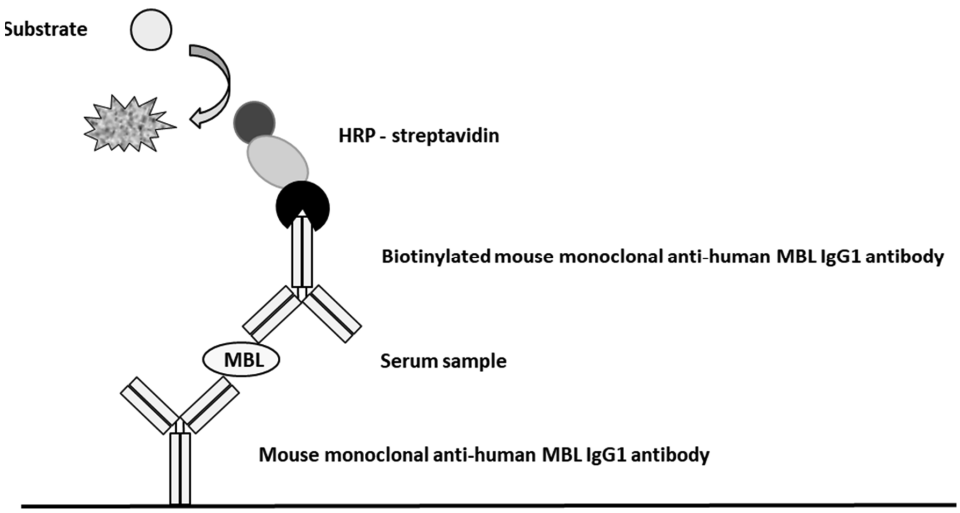


Figure 6. A schematic drawing of the sandwich ELISA. The plate is coated with mouse monoclonal anti-human MBL IgG1 used as the capture antibody. When human serum is added, biologically active MBL (antigen) binds to the capture antibody. Biotinylated mouse monoclonal anti-human MBL IgG1 antibody binds specifically to MBL. HRP conjugated in streptavidin recognizes biotin of the detecting antibody, and, in the presence of a substrate, HRP enzyme produces the detectable signal. HRP=horseradish-peroxidase, MBL=mannose-binding lectin, IgG=immunoglobulin G.

4.8 Statistical analyses (I-IV)

A generalized estimating equation (GEE) with a binomial error distribution and logit link function was used to model the probability of an adult *I. ricinus* tick to be positive for Bbsl in comparison with an adult *I. persulcatus* (I). Larvae and nymphs were ignored because of their low sample sizes. The analysis was restricted to regions of sympatric occurrence of *I. ricinus* (n=527; 393 females and 134 males) and *I. persulcatus* (n=885; 658 females and 227 males) in order to exclude the effect of dissimilar environments (e.g., weather conditions or distance to water areas) on the pathogen prevalence in ticks. This was done in practice by filtering the data according to the N coordinate of the southernmost *I. persulcatus* and northernmost *I. ricinus*. The species and sex of the tick were fixed explanatory factors, while the shipment identification was set as a clustering factor. Results were presented as marginal means with 95% confidence intervals (CIs).

The annual LB incidence rates and the age- and sex-specific average annualized LB incidence rates were calculated using data from the National Population Information System as denominators (II). Poisson regression was used for the time trend analysis by the HDs.

In study III, the Bbsl seroprevalence results were merged with the general questionnaire and health data and all statistical analyses were adjusted for the stratified cluster design and sampling weights. Weighted seroprevalence estimates were reported for the whole country and stratified for variables of sex, age, university hospital regions, educational level, household size, and profession. Using logistic regression, odds ratios (OR) and 95% CIs were estimated to determine the association between potential risk factors and Bbsl seropositive status. Variables with a p-value ≤ 0.2 in univariable analyses were stepwise added to the multivariable model, and those variables showing a significant improvement were selected for the final model. The association between Bbsl seropositive status and chronic diseases was assessed using the final model and adjusted for confounders of sex, age, and hospital region.

Optimal serum concentration cut-off values were determined using 10% and 40% cut-offs of the MBL pathway function (IV). The MBL pathway function was used as a binary response and serum MBL concentration as an independent variable in receiver operating characteristic (ROC) analyses. Minimum sensitivity set to 80% yielded the cut-off values of 445 and 787 ng/ml with specificities of 97.3% and 93.8%, respectively. The effect of age on serum MBL concentration, controlling for groups, was analyzed using the analysis of covariance (ANCOVA). A square-root transformation was applied because the distribution of serum MBL concentration was skewed. Model fit was confirmed with Q-Q plotted Pearson residuals. Difference in the serum MBL concentrations between the genders and between the

LB patients and non-LB controls were investigated using logistic regression with logit link. There were two parallel models for MBL cut-off values for LB positivity as a response and the MBL cut-off, age, gender, and age and gender interaction as independent variables. Results were presented as ORs and 95% CIs.

In studies I and IV, the data were managed using Microsoft Excel 2013 (Redmond, WA, USA), and statistical analyses were performed with SAS statistical software version 9.4 (SAS Institute, Cary, NC, USA). Figures in study IV were drawn with R 3.1.0 (Foundation for Statistical Computing, Vienna, Austria). In studies II and III, Stata v. 14.2 (Stata Corp., TX, USA) was used for data management and statistical analyses.

In all analyses, p-values <0.05 were considered statistically significant.

4.9 Ethics

Register data derived from three national health care registries (NIDR, Hilmo, and Avohilmo) in study II were handled anonymously, and only age and sex were recorded from personal data. Research authorization to access data was obtained from NIHW.

The serum samples used in study III were derived from a large health examination survey (Health 2011) where a representative sample (n=2 000) of the general adult population in Finland was collected. The Health 2011 Survey has ethical approval obtained from the Coordinating Ethical Committee of the Helsinki and Uusimaa Hospital Region. All blood donors of the survey signed informed consent allowing the use of their samples and data in medical and public health research. The samples are stored in the NIHW Biobank with the approval of the Coordinating Ethics Committee of Helsinki University Hospital and the Ministry of Social Affairs and Health. For study III, approval to use the samples of Health 2011 Survey was obtained directly from the Health 2011 surveillance research group (NIHW).

The serum samples used in study IV were collected with informed consent as part of a routine clinical practice from individuals who were clinically suspected to have LB. All samples were handled anonymously and no other clinical data except age and gender, were used. Because the research was not medical research according to the Finnish Medical Research Act (No. 488/1999) (available at: http://www.finlex.fi/en/laki/kaannokset/1999/en19990488 [Accessed: 23rd July 2017]), separate approval from the local Ethics Committee for use of the samples in study IV was not needed. According to Finnish Medical Research Act (No. 488/1999), the statement of Ethics Committee is only needed in research involving medical intervention.

5 RESULTS

5.1 The Tickbank

5.1.1 Tickbank characteristics (I)

Altogether, 17 936 ticks could be identified morphologically as *I. ricinus* (n=14 133; ~80%) or *I. persulcatus* (n=3 803; ~20%). The vast majority of the ticks were adults, and of these, over 2/3 were females. The sex distribution of adult ticks was similar in both species. More young developmental stages (larvae and nymphs) were among *I. ricinus* samples in comparison with *I. persulcatus* samples (5.5% vs. 1.1%, respectively).

The most commonly reported host was a dog for both tick species (54.2% for *I. ricinus* and 62.2% for *I. persulcatus*). More *I. persulcatus* than *I. ricinus* were collected from humans (19.7% vs. 14.5%, respectively), whereas *I. ricinus* was collected more often from cats (30.3% vs. 17.3%).

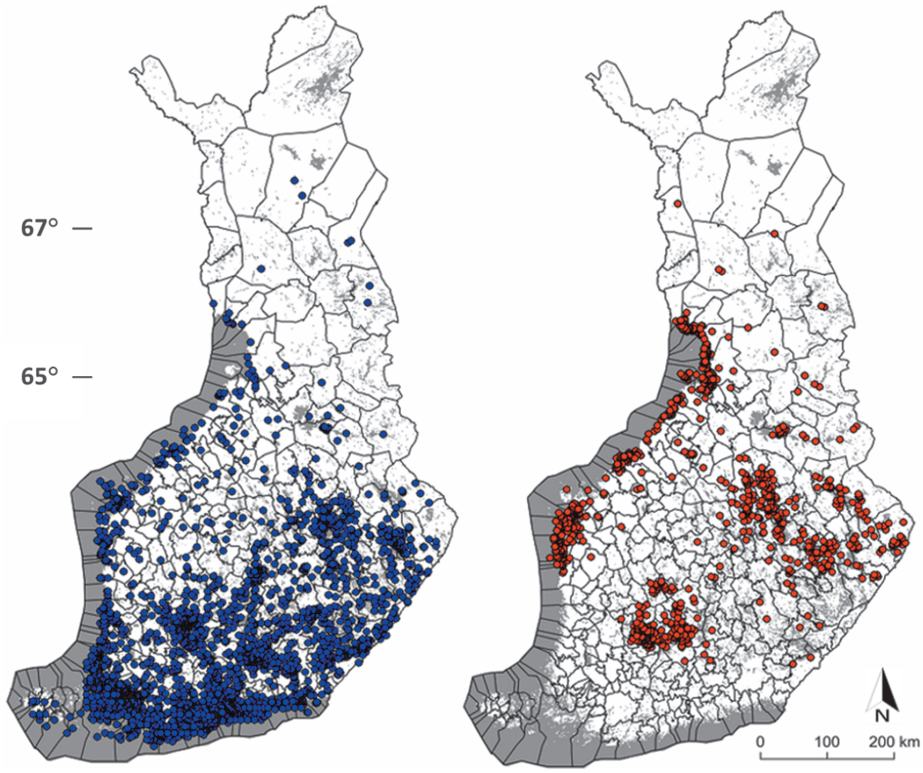


Figure 7. The geographical distribution of *Ixodes ricinus* and *I. persulcatus* in Finland based on the coordinates of 17 603 ticks collected in 2015. Blue dots indicate the collection points of *I. ricinus* (n=13 847) and red dots the collection points of *I. persulcatus* (n= 3 756). Modified from the original publication I.

5.1.2 Geographical distribution of I. ricinus and I. persulcatus (I)

Maps of the geographical distribution of *I. ricinus* and *I. persulcatus* were drawn based on the coordinates of 17 603 ticks (13 847 *I. ricinus* and 3 756 *I. persulcatus*) (Figure 7). The distribution of ticks largely covered central and southern Finland, especially concentrating on the proximity of large water areas: the Finnish Lakeland (in south-eastern Finland) and coastal areas. *I. ricinus* ticks were collected extensively over southern and eastern Finland and the coastal areas, whereas *I. persulcatus* seemed to be clustered in three distinct areas: on the coast of the Gulf of Bothnia, in eastern Finland, and in the middle of southern Finland. Both tick species were received from northern Finland north of latitude 65ºN, but a vast majority of these ticks were *I. persulcatus* (760/784; 97%). The northernmost collection sites were at latitudes of 67ºN in Lapland.

5.1.3 The prevalence of Bbsl in Finnish ticks (I)

The prevalence of Bbsl was analyzed from a total of 2 038 ticks, of which 1 044 were *I. ricinus* and 994 *I. persulcatus*. In total, 16.9% (n=345) of the screened tick DNA samples were Bbsl positive. The prevalence was somewhat higher in *I. persulcatus* compared with *I. ricinus* (19.8% vs. 14.2%, respectively). From adult ticks, 17.1% (332/1 945) were Bbsl positive, while the prevalence of nymphs was 14.3% (13/91). No larvae were infected. A significantly higher probability of a positive Bbsl finding was detected for adult *I. persulcatus* (the estimated marginal mean was 0.196 [95% CI: 0.166–0.232]) than for adult *I. ricinus* (0.137 [0.106–0.174]) (Wald statistics, species: χ^2=5.67, DF=1, p=0.017) ticks in the sympatric region of these tick species. Between the genders of either species, no differences in the prevalence of Bbsl were detected (sex: χ^2=1.03, DF=1, p=0.311; species × sex: χ^2=0.03, DF=1, p=0.872). The distribution map of the positive Bbsl ticks is presented in Figure 8A.

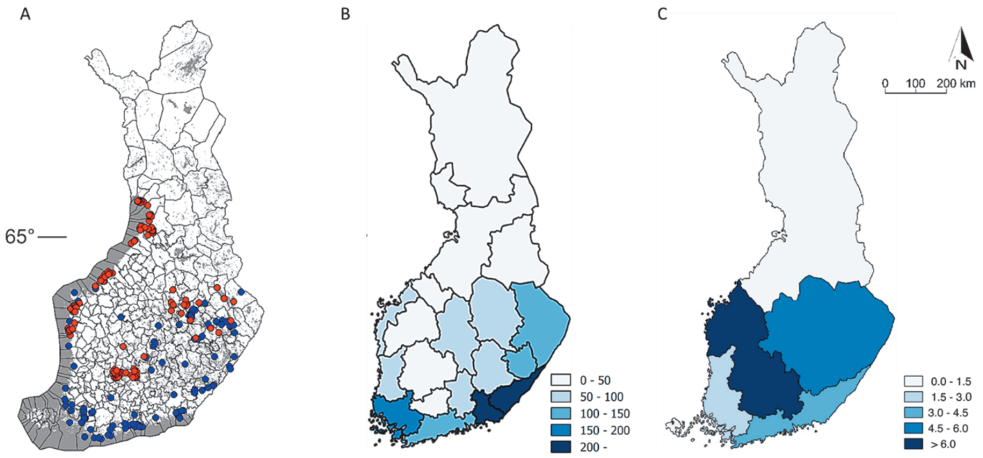


Figure 8. A) Distribution of the ticks that were positive for Bbsl. Blue dots indicate the collection points of *I. ricinus* (n=148) and red dots indicate the collection points of *I. persulcatus* (n=197). B) Mean annual LB incidences per 100 000 population by hospital districts in 2011−2015. The clinically diagnosed and microbiologically confirmed LB cases are added up with the correction considering that on average, 70% of the clinically diagnosed LB cases are correctly coded in Avohilmo. C) The geographical distribution of Bbsl seroprevalence (%) in Finnish adults by university hospital region. Modified from the original publications I, II, and III.

5.1.4 Other tick-transmitted pathogens in Finnish ticks: TBEV and B. miyamotoi (I)

In addition to Bbsl, the prevalences of TBEV and *B. miyamotoi* were analyzed from the subset of 2 038 ticks. In total, 32 (1.6%) of the screened RNA samples were positive for TBEV. A higher prevalence was observed in *I. persulcatus* compared with *I. ricinus* (3.0% vs. 0.2%, respectively). Apart from one *I. persulcatus* nymph, all TBEV positive samples were adults. TBEV positive ticks were received from the coastal areas in Bothnian Bay, eastern Finland, and south-central Finland.

B. miyamotoi was found in a total of six ticks, all of which were adults. Two were *I. ricinus* (0.2%) and four were *I. persulcatus* (0.4%). Positive ticks were received from south-western Finland, central Finland, and the coast of Bothnian Bay.

Co-infections were detected with TBEV and Bbsl (eight *I. persulcatus* ticks: two males and six females) and with *B. miyamotoi* and Bbsl (two *I. persulcatus* ticks: male and female).

5.2 Epidemiology of LB

5.2.1 Increased incidence of LB (II)

From 1995 to 2015, a total of 23 028 microbiologically confirmed LB cases were reported to NIDR. The annual number of cases increased significantly ($p<0.05$) from a few hundred (~7–10/100 000 population) in the 1990's to over 1 900 (35/100 000 population) in 2015 (Figure 9). Between 2011 and 2015, a total of 15 386 clinically diagnosed LB cases were identified in Avohilmo. The average annual number of cases was approximately 3 000–3 500, with an increasing trend from 44/100 000 population to 65 during these past five years ($p<0.05$). By summing microbiologically confirmed and clinically diagnosed LB cases together, the total number of cases was estimated to be 5 019 (93/100 000 population) in 2011 and 6 957 (127/100 000 population) in 2015. These calculations include the assumptions that 1) the number of clinically diagnosed LB cases does not substantially overlap with the microbiologically confirmed cases, and that 2) on average, 70% of all LB diagnoses are correctly coded with the ICD-10 code in Avohilmo by the general practitioners.

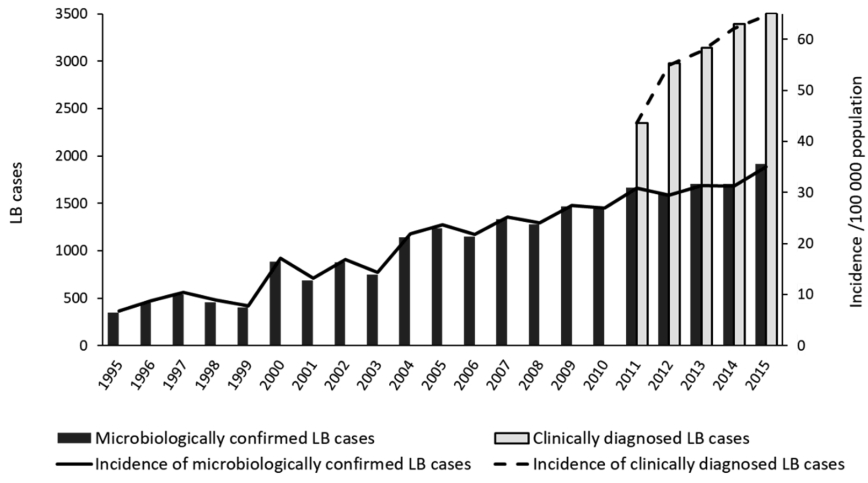


Figure 9. Number and incidence rates of microbiologically confirmed (n=23 028) and clinically diagnosed LB cases (n=15 386) in Finland, 1995−2015. LB cases in occupational and private health care are not included in clinically diagnosed LB cases. Modified from the original publication II.

5.2.2 Geographical distribution of LB incidence and LB seroprevalence (II, III)

During the past five years (2011–2015), the highest average annual incidences of microbiologically confirmed LB cases were reported in south-eastern Finland, ranging from 45/100 000 population (South Karelia) to 55 (Kymenlaakso) and in the Southwest Finland 51/100 000 population. In the Åland Islands, the incidence was 1 740/100 000 population. The lowest annual incidences with 1–5 microbiologically confirmed LB cases per 100 000 population were reported in northern and north-eastern Finland as well as in the middle of southern Finland during 2011–2015. The average annual incidence of the whole country was 32/100 000 population.

During the same years, the highest average annual incidences of clinically diagnosed LB cases were also reported in Kymenlaakso (137/100 000 population), South Karelia (168/100 000 population), and the Southwest Finland (89/100 000 population). In the Åland Islands, the average annual incidence of clinically diagnosed LB cases was 910/100 000 population. The countrywide average annual incidence was 57/100 000 population.

To further provide average incidence rates for the total annual number of LB cases by HDs during 2011–2015, both the clinically diagnosed and microbiologically confirmed LB cases were added up with the correction taken into consideration in clinically diagnosed cases (on average, 70% of the LB cases are correctly coded in Avohilmo). The HD-specific average annual incidence rates in 2011–2015 reflecting the current epidemiological situation of LB in Finland are presented in Table 5 and Figure 8B.

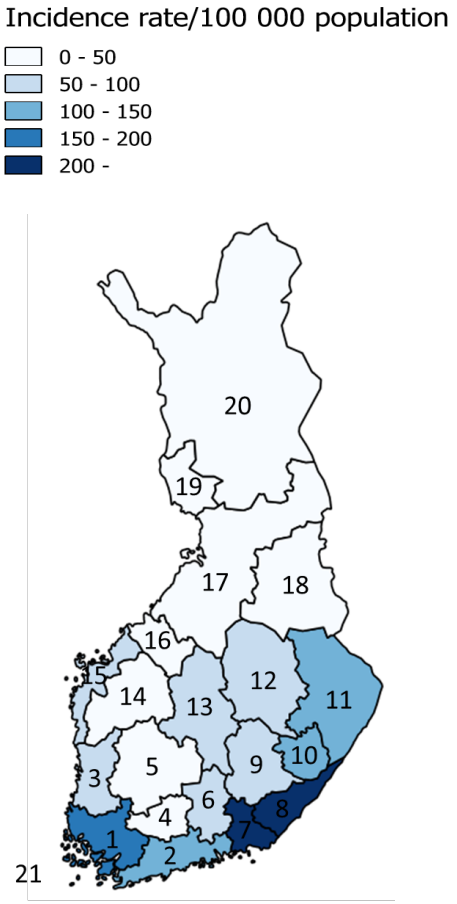
Figure 8C shows the geographical distribution of Bbsl IgG seroprevalence in Finnish adults ≥29 years by university hospital region. Of 2 000 tested serum samples, 86 (4.3%) were positive for IgG antibodies against Bbsl, resulting in a weighted seroprevalence of 3.9% (95% CI: 3.0–5.1). In univariate analysis, significantly higher seroprevalence was observed in the southern, central, and eastern university regions in comparison with the northern region. The western region also had a slightly higher seroprevalence than the northern region but the difference was not statistically significant.

5.2.3 Time trend of LB incidence by hospital districts (II)

Changes in the geographical distribution of LB incidence over time in study II were analyzed using both the microbiologically confirmed LB cases in NIDR and

Table 5. The average annual incidence rates for the total number of LB cases by HDs in 2011–2015. The clinically diagnosed and microbiologically confirmed LB cases are added up with the correction considering that on average 70% of the clinically diagnosed LB cases are correctly coded in Avohilmo.

	Hospital district	Total number of LB cases /100 000 population	No of clinically diagnosed LB cases/ 100 000 population	No of microbiologically confirmed LB cases/ 100 000 population
1	Southwest Finland	178.2	89.0	51.0
2	Helsinki and Uusimaa	128.8	66.1	35.2
3	Satakunta	54.5	28.0	14.4
4	Tavastia Proper	30.3	18.4	4.1
5	Pirkanmaa	28.4	18.0	2.7
6	Päijät-Häme	54.7	30.1	11.7
7	Kymenlaakso	250.4	136.9	54.8
8	South Karelia	284.5	167.8	44.7
9	Southern Savonia	87.0	52.4	12.2
10	Eastern Savonia	127.8	79.3	14.8
11	North Karelia	109.6	65.2	16.4
12	Northern Savonia	95.8	57.2	14.1
13	Central Finland	88.0	54.9	9.4
14	Southern Ostrobothnia	27.3	17.8	1.8
15	Vaasa	94.7	40.5	36.8
16	Central Ostrobothnia	49.4	27.6	10.0
17	Northern Ostrobothnia	9.3	5.2	1.8
18	Kainuu	8.5	4.2	2.6
19	Länsi-Pohja	17.8	9.6	4.0
20	Lapland	13.1	6.9	3.2
21	The Åland Islands	3040.2	909.9	1740.7
	The whole country	112.7	56.6	31.6



clinically diagnosed LB cases in Avohilmo. Four different time periods (1995–1999, 2000–2004, 2005–2009, and 2010–2014) were used in the calculations of the average annual incidences of the microbiologically confirmed LB cases by HDs. The incidence of microbiologically confirmed LB cases increased significantly ($p<0.05$) in most of the HDs (15/21, 71.4%) over time. The most notable increasing trend was observed in western and south-eastern Finland.

During 2011–2014, when Avohilmo data were available for study II, the incidence of clinically diagnosed LB cases increased significantly in eight HDs, particularly in southern and central Finland.

5.2.4 Demographic characteristics of LB cases (II, III)

Demographic characteristics of LB cases were examined using both the register and seroprevalence data. Clinically diagnosed LB cases in Avohilmo represent mainly EM infections that are diagnosed in primary health care, whereas microbiologically confirmed LB cases in NIDR and the seroprevalence data of Finnish adults (Health 2011 serum samples) represent the disseminated infections.

Of the 15 386 clinically diagnosed LB cases in Avohilmo, 9 142 (59.4%) occurred in females. Apart from children between 5 and 14 years and the elderly over 80 years, female preponderance was observed across all age groups (Figure 10A). In the age groups between 60 and 79 years, annual LB incidence was distinctively higher among females than among males (on average 148/100 000 population vs. 106/100 000 population, respectively).

Between 1995–2015, a total of 23 028 microbiologically confirmed LB cases were reported to NIDR. Slightly more cases were women (n=12 372; 53.7%). The highest incidence occurred in females of 60–69 years old and in males over 70 years (Figure 10B). There were no significant differences in incidences between genders across the age groups, except the notable preponderance of males over 70 years. In general, the age distribution was similarly bimodal as in the clinically diagnosed LB cases, with the peaks occurring in the age groups of 60–79 years and a minor peak in children aged 5–9 years old.

Of 2 000 serum samples that were used to study the Bbsl seroprevalence in Finnish adults, 86 were positive for IgG antibodies against Bbsl. From these, 51 (59.3%) were males. The weighted seroprevalence among males was significantly higher in comparison with females (adjusted OR: 1.91, 95% CI: 1.21–3.04, p=0.005), and a clear increase of age-related seroprevalence was observed.

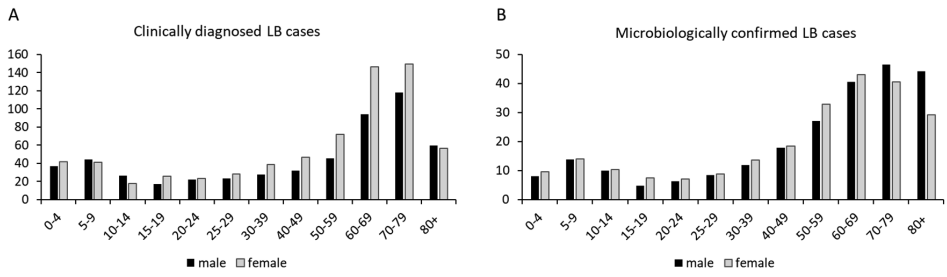


Figure 10. A) Incidence rates of clinically diagnosed LB cases (n=15 386) by age and gender, Finland, 2011−2015. LB cases in occupational and private health care are not included in clinically diagnosed LB cases. B) Incidence rates of microbiologically confirmed LB cases (n=23 028) by age and gender, Finland, 1995−2015. Modified from the original publication II.

5.2.5 Seasonality of ticks and LB infections (I, II)

The questing period of *Ixodes* ticks begins in March and takes place through the Finnish summer months (Gray *et al.* 2009). In study I, most of the ticks were collected in May (Figure 11). The collection period of *I. ricinus* continuing through the summer and early autumn was notably longer than that of *I. persulcatus*. The latter was mainly collected from April to June (98.1% of the ticks) and was no longer found in autumn.

Clinically diagnosed LB cases peaked in July and August, and most of the cases (~75%) occurred between June and September (Figure 11). Only a few cases were reported to Avohilmo during the Finnish winter from November to April. Microbiologically confirmed LB cases were notified to NIDR through the year, although a pronounced peak was noticed in September following the clinically diagnosed cases around 1–2 months. Year-to-year variation in seasonality was minor among microbiologically confirmed and clinically diagnosed cases.

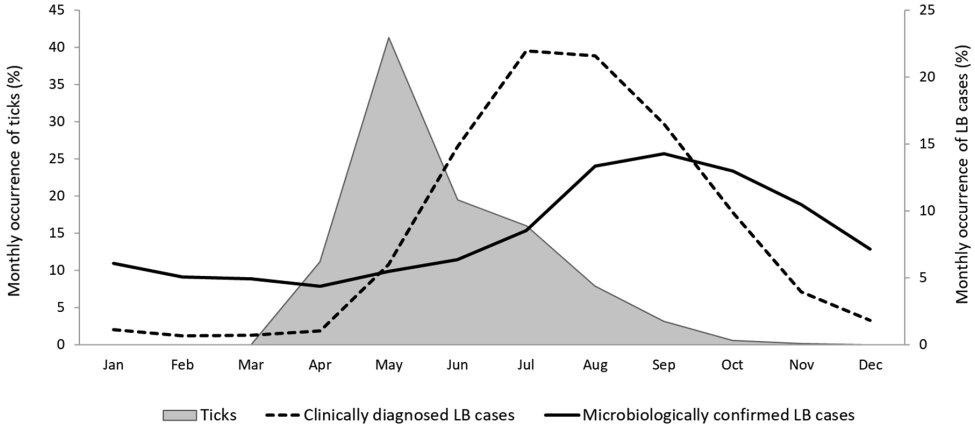


Figure 11. Monthly occurrence of ticks (*I. ricinus* and *I. persulcatus*) received via the tick collection campaign and seasonal distribution of clinically diagnosed and microbiologically confirmed LB cases, Finland. The tick data covers the year 2015, clinically diagnosed LB cases cover 2011−2015, and the microbiologically confirmed LB cases cover 1995−2015. Modified from the original publications I and II.

5.2.6 Clinical manifestations of LB (II)

The age- and sex-specific distribution of LB cases based on Hilmo database was similar to that based on NIDR, as expected. Hilmo represents the LB cases diagnosed in inpatient healthcare, and thus, includes those cases that are most likely confirmed by laboratory testing.

Hilmo data were used to study the clinical manifestations of the LB cases in study II. During 1996–2014, 968 LNB cases and 450 LA cases were identified in Hilmo. A few dozen LNB cases were notified yearly with a peak of 129 cases (2.4/100 000 population) in 2011. The number of LA cases ranged from around 10 to 40 and the incidence never exceeded 1.0/100 000 population. Of all annual LB cases in Hilmo, LNB represented around 5–12% while LA made up 2–13% depending on the year.

The bimodal age-specific distribution of LNB and LA incidences with peaks occurring in children of 5–14 years and adults 60–69 years of age were seen in females and males. No significant differences in the LNB or LA incidences by HDs were observed.

5.3 Susceptibility to LB

5.3.1 Risk factors for LB (III)

The personal data related to Health 2011 serum samples included a vast amount of data regarding the individual's health and socioeconomic status. Different determinants were reviewed as possible factors predisposing to or protecting from LB. Being a part-time employee, a pensioner, and having a quite good health status were associated with LB seropositivity, whereas a higher level of education and a household size of 3–5 persons seemed to correlate negatively to LB seropositivity in univariate analysis. However, when adjusted for age, sex, and hospital region, none of these associations were statistically significant. None of the analyzed chronic diseases (e.g., cancer, or pulmonary, skin, rheumatic, or autoimmune diseases) was positively associated with LB seropositivity.

5.3.2 Determination of the cut-off values for diminished and deficient MBL serum concentration (IV)

MBL serum concentration and MBL pathway function of 201 serum samples were plotted against each other to determine the MBL cut-off concentrations for the impaired MBL pathway function. Diminished MBL pathway function (<40%) was associated with MBL concentration <787 ng/ml, and deficient MBL pathway function (<10%) was associated with MBL concentration <445 ng/ml with the specificities of 93.8% and 97.3%, respectively, when the minimum sensitivity was set to 80%. These MBL concentrations (<787 and <445 ng/ml) were used as the cut-off concentrations for deficient MBL pathway function (IV). Importantly, MBL pathway functions were not analyzed from the actual serum samples (350 LB patients and 350 non-LB controls) used in study IV.

5.3.3 Deficient MBL pathway of complement in LB (IV)

A declining trend in the MBL concentration with age was observed in both LB patient group (β=-0.17, p=0.002) and the non-LB control group (β= -0.11, p=0.023) (Figure 12). Low MBL serum levels indicated by the MBL concentration <787 ng/ml were found in ~25–30% of the non-LB control samples through the age groups (0–10, 11–20, 21–30, and so forth). Several samples had a serum con-

centration exceeding the upper detection limit of the assay (6 400 ng/ml). By comparison, only a few samples of the LB patients exceeded this concentration. No difference in the MBL concentrations between genders was observed (p=0.917).

Using the determined cut-off concentrations, the diminished MBL pathway function occurred significantly more frequently among the LB samples (145/350; 41.4%) than among the non-LB control samples (96/350; 27.4%) (p=0.003; OR 1.67; 95% CI: 1.19–2.34) when age, gender, and age and gender interaction were controlled for the statistical analyses (Figure 12). Similarly, deficient MBL pathway functions observed in 92 (26.3%) of the LB samples and 60 (17.1%) of the control samples were also significantly different (p=0.036; OR 1.51; 95% CI: 1.03–2.23). In general, significantly lower MBL concentrations were measured in LB samples compared with control samples. The median MBL concentration was 1 088 ng/ml (interquartile range 418–2 166 ng/ml) in LB samples and 1 988 ng/ml (interquartile range 687–4 220 ng/ml) in control samples (p<0.0001) (Figure 13).

In *recom*Line Borrelia IgG line immunoblot, IgG antibodies against *B. afzelii* p18 antigen (Decorin binding protein A) were frequently detected reactivity in the assay. This reflects the supposition that *B. afzelii* is the most common genospecies in Finland. Among the LB patients, antibodies against *B. afzelii* p18 were present in 87/190 (45.8%). MBL pathway function was diminished in 40 of these 87 patients (46.0%) and deficient MBL pathway function in 31/87 (35.6%). Thus, the diminished/deficient MBL pathway function was even more frequent in the LB patients who had *B. afzelii* p18 IgG antibodies compared with all LB patients (46.0% vs. 41.4%, and 35.6% vs. 26.3%, respectively). In comparison with the non-LB controls, significant differences in the frequency of diminished (46.0% vs. 27.4%, p=0.002, OR 2.34, 95% CI: 1.38–3.97) and deficient (35.6% vs. 17.1%, p=0.026, OR 1.96, 95% CI: 1.09–3.52) MBL pathway functions were observed. No difference in the MBL concentration between genders in this subset of patients was detected (p=0.703). In addition, the age-adjusted median MBL concentration was lower in those LB patients who had *B. afzelii* p18 IgG antibodies in comparison with the controls (968 vs. 1 988 ng/ml, p<0.0001). (Figure 13)

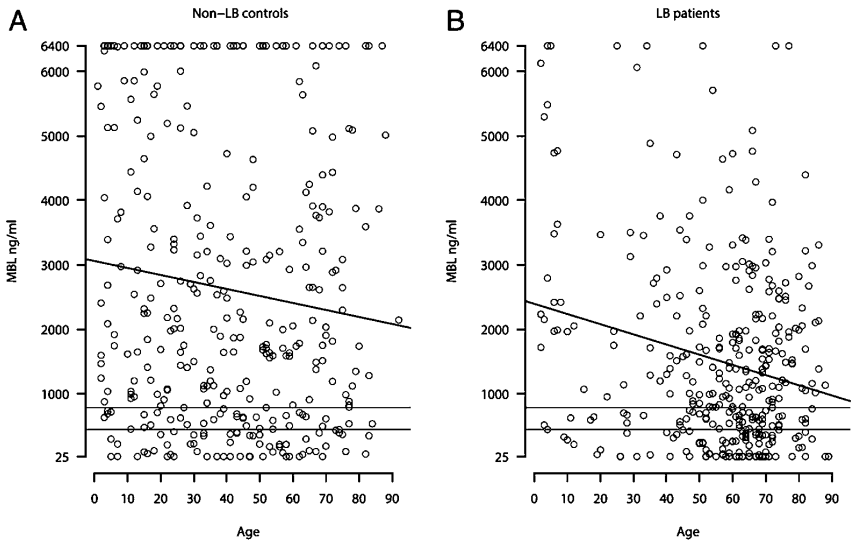


Figure 12. MBL concentrations in sera of non-LB controls and LB patients. (A) In the non-LB controls, diminished MBL pathway function (<787 ng/ml) was observed in 27.4% of subjects, and deficient MBL pathway function (<445 ng/ml) was observed in 17.1% of subjects. Notably, diminished serum MBL levels were found in ∼25–30% of the controls through the age groups (0–10, 11–20, 21–30, and so forth). Several samples had a serum MBL concentration exceeding the upper limit of the assay (6 400 ng/ml). (B) In the LB patients, the frequency of diminished MBL pathway function was 41.4%, and the frequency of deficient MBL pathway function was 26.3%. The MBL concentration was above the upper limit of the assay (6 400 ng/ml) in only a few samples. Adapted from the original publication IV and reprinted with permission from The American Association of Immunologists, Inc.

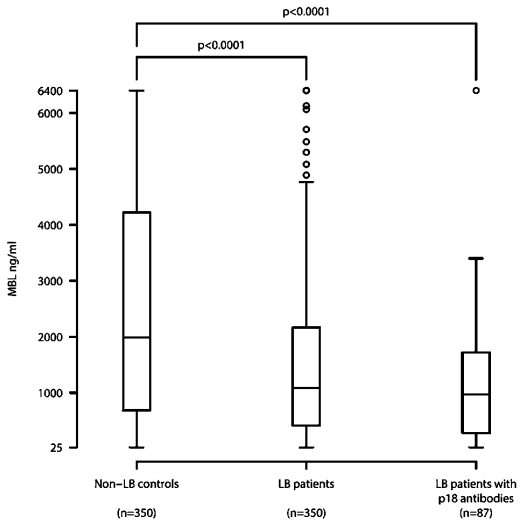


Figure 13. MBL concentrations of the LB patient samples, non-LB control samples, and the subset of LB patients with IgG antibodies against the *B. afzelii* p18 antigen. The median MBL concentration was 1 988 ng/ml in non-LB samples, 1 088 ng/ml in LB patient samples, and 968 ng/ml in the samples from LB patients with *B. afzelii* p18 IgG antibodies. In general, the serum MBL concentrations were significantly ($p < 0.0001$) lower among the LB samples than in non-LB controls. The LB samples with IgG antibodies against *B. afzelii* p18 also had significantly ($p < 0.0001$) lower serum MBL concentrations in comparison with non-LB controls. Adapted from the original publication IV and reprinted with permission from The American Association of Immunologists, Inc.

6 DISCUSSION

6.1 Geographical distribution of ticks and increasing LB incidence

The last nationwide distribution map of *Ixodes* ticks in Finland was presented based on a survey made in the late 1950s (Öhman 1961). At that time, *I. persulcatus* was not found in Finland and the northernmost distribution border of *I. ricinus* was placed around Vaasa and Northern Savonia. According to our study (I), the distribution border of ticks has since shifted 200–300 km northwards, *I. persulcatus* has widely established itself in Finland, and *I. ricinus* has spread into new areas especially along the coast of Bothnian Bay.

The similar northwards shift in tick distribution as well as the increased tick abundance have been noted in other northern European countries and Canada (Tälleklint and Jaenson 1998, Lindgren *et al.* 2000, Ogden *et al.* 2006, Jore *et al.* 2011, Jaenson *et al.* 2012, Bugmyrin *et al.* 2013, Sormunen *et al.* 2016a). At the same time with observed changes in tick populations, the increasing trend in LB incidence has been reported in several European countries, various states of the US, and Canada (Mead 2015). Also in our study (II), the increasing incidence of LB during the past two decades was demonstrated by the increased number of microbiologically confirmed LB cases from a few hundred in the late 1990s to approximately 1 900 in 2015. The trend of increasing incidence seemed even steeper in clinically diagnosed LB cases between 2011 and 2015, further confirming this trend in past recent years.

One frequently suggested driving force behind the above-mentioned changes in tick distribution and increasing LB incidence is global warming affecting the ticks and their host animals. A study conducted in primary health care clinics in southern Sweden in 2006 reported an increased incidence of EM after milder winters and during warm, humid summers (Bennet *et al.* 2006). Another study from the same country observed an increased incidence of TBE associated with milder winters, the early arrival of spring, and an extended autumn season (Lindgren and Gustafson 2001). Climatic factors have been suggested to affect the ticks and their host animal survival over winter, tick population density, questing activity, and the developmental rate, as well as human exposure to tick bites in the summertime (Bennet *et al.* 2006, Gray *et al.* 2009, Lindgren *et al.* 2000, Jaenson and Lindgren 2011, Jaenson *et al.* 2012, Medlock *et al.* 2013).

In Finland, roughly every fifth tick encountered in nature is *I. persulcatus* according to our tick collection (I). The species seems to be clustered in three different geographical areas, and in the north, it is even more abundant than *I. ricinus*. This

could be due to better cold-resistance of *I. persulcatus*, as suggested in previous studies (Tokarevich *et al.* 2011). The southern coast of Finland, however, was exclusively colonized by *I. ricinus*. We hypothesized that *I. persulcatus* as a later arrival in Finland has not succeeded in establishing itself in those areas where *I. ricinus* was already widely present. These two species can crossbreed, but the number of produced eggs is low and offspring sterile (Balashov *et al.* 1998), which may hinder the formation of stable *I. persulcatus* populations in the southern coast of Finland. Preferences in vegetation, landscape, and other biotopic factors, and different seasonal activity patterns may have an effect as well.

Because the ticks were collected with the help of citizens instead of professional scientists doing traditional fieldwork, most of them were collected from humans and their companion animals and not from natural habitats of ticks. In a previous study conducted in southern Karelia (Russia), the collection method (transect dragging, i.e., pulling a cloth through leaf litter or vegetation vs. from pets) considerably affected the tick species composition (*I. ricinus* vs. *I. persulcatus*). In a given geographical region, the proportion of *I. ricinus* was higher when the ticks were collected from pets instead of by dragging (Bugmyrin *et al.* 2013). However, the distributional areas of *I. ricinus* and *I. persulcatus* in our study were in many parts so distinct from each other that the bias caused by the collection method is hardly sufficient to negate the main distributional areas found in the study. Furthermore, in recent studies conducted by dragging in south-western Finland, the only tick species present has been *I. ricinus*, further confirming the absence of *I. persulcatus* in the southern coast of Finland (Sormunen *et al.* 2016a, Sormunen *et al.* 2016b).

Likely due to better detectability and longer questing activity of adult female ticks in comparison with nymphs and larvae, the Tickbank contained mainly adult ticks, although their proportion in nature is a fraction of that of the number of larvae and nymphs (Sormunen *et al.* 2016a). Thus, for ecological research the collection is biased. Instead, it reflects the areas where human-tick encounters occur, which is further indicated by the substantially overlapping distributional maps of ticks and LB cases in Finland (Figure 7 and Figure 8). Correspondingly, LB incidence is low in those HDs where ticks are sparsely distributed, like in northern Finland.

In addition to the increased abundance of vector ticks, there might be also other reasons having influence on the increasing LB incidence. Increased awareness and interest in tick-borne infections among health care professionals and the public have undoubtedly increased the diagnostic activity and surveillance of LB. Improved laboratory diagnostic methods for LB might have had an influence on the increased number of LB diagnoses as well. However, in our study II, we could not identify any changes in the laboratory methodology of Finnish microbiological la-

boratories performing LB diagnostics (n=8) that could have explained the increased incidence of microbiologically confirmed LB cases in Finland. In fact, the adoption of a 2-tier approach to Bbsl serologic testing has most likely increased the specificity of LB diagnostics.

6.2 Pathogen prevalence in ticks

The overall prevalence of Bbsl was 16.9% (I) with a higher prevalence detected in *I. persulcatus* in comparison with *I. ricinus* (19.8% vs. 14.2%, respectively). The probability of a positive Bbsl finding was significantly higher in *I. persulcatus* than in *I. ricinus* when the analysis was restricted to the adult ticks of the sympatric region. However, in light of LB incidence, it hardly matters if the tick encountered by the human is *I. ricinus* or *I. persulcatus*. Although somewhat higher infection rates of certain tick-borne pathogens, such as Bbsl and TBEV, have been reported on *I. persulcatus* in previous studies (Geller *et al.* 2013, Katargina *et al.* 2013) and in our study, both species are equally able to transmit pathogens to humans, and to date, no evidence of either of the species being a more efficient vector exists. Furthermore, despite the slightly higher overall prevalence of Bbsl in *I. persulcatus* ticks, great temporal and geographical variation among tick populations occur. In a study conducted in south-western Finland in 2015, 23.5% of adult *I. ricinus* ticks were found to be Bbsl positive (Sormunen *et al.* 2016b), whereas an earlier study conducted in the recreational parks in Helsinki in 1999 reported a prevalence of up to 55% (Junttila *et al.* 1999). Our result, 14.2% of *I. ricinus* positive for Bbsl, represents the average of the whole country.

Bbsl species differ in their pathogenicity. The very recent results of molecular typing of Bbsl species suggest intraspecies differences (e.g., among *B. afzelii* genotypes) in the pathogenicity of Bbsl in addition to interspecies differences (e.g., *B. afzelii* vs. *B. lusitaniae*) (Coipan *et al.* 2016). The number of Bbsl genotypes circulating in ticks is far higher than the number of genotypes isolated from human samples, thus suggesting that not all the Bbsl genotypes within a given genospecies (e.g., *B. afzelii*) are equally pathogenic. This is especially interesting when looking at the low LB incidence area in the middle of southern Finland (Pirkanmaa and Proper Tavastia), which still seems to be an area of high tick occurrence (Figure 8B and Figure 7). Could an explanation for the lack of LB cases in this area be in the population of ticks carrying less pathogenic Bbsl genospecies and genotypes? Or perhaps the Bbsl prevalence of ticks around this area is for some reason lower than elsewhere in the country. Most likely a more plausible explanation can be given based on the reporting differences of LB in the HDs. This seems likely when

the discrepancy between the low LB incidence rates versus high Bbsl seroprevalence results in the middle of southern Finland are noted in Figure 8. However, we did not determine the genospecies nor the genotypes of Bbsl strains derived from ticks in our study (I), which is worthy of attention in the future. Additionally, with greater regional subsets of ticks, geographical differences in Bbsl prevalences among tick populations could be investigated. A tick might also be simultaneously co-infected with two or more Bbsl species, and patients may become double infected by the bite of a single tick (Oksi *et al.* 1995).

The 1.6% overall prevalence of TBEV in our study (I) corresponds to the prevalences of 0.2–2.0% reported in questing ticks in TBE-endemic areas elsewhere in Europe (Süss *et al.* 2002). The distribution of TBEV in nature is patchy and the annual prevalence in ticks can vary considerably (Bormane *et al.* 2004). From three TBEV subtypes, especially Eur-TBEV has a highly focal distribution, whereas Sib- and FE-TBEV seem to cover the range of *I. persulcatus* more widely (Lindquist and Vapalahti 2008). The latter two are mainly transmitted by *I. persulcatus*, whereas the principal vector for Eur-TBEV is *I. ricinus*. Thus, the coincidence related to the sparse distribution of TBEV could explain why none of the ticks collected from the southern and south-western coasts of Finland were positive for the virus. Nevertheless, this contradicts the fact that the majority of human TBE cases occur along the southern coast of Finland (Tonteri *et al.* 2015). Identifying the subtypes of the TBEV positive findings could shed more light on this issue, especially because such a remarkable majority of positive ticks were *I. persulcatus* (30 of 32 TBEV positive ticks).

Eight ticks (0.4%), all *I. persulcatus*, were co-infected with Bbsl and TBEV. Co-infections of LB and TBE are rather rare, but patient cases have been reported in Finland, Latvia, Germany, Slovenia, and Russia (Kristoferitsch *et al.* 1986, Oksi *et al.* 1993, Cimperman *et al.* 1998, Amosov *et al.* 2000, Cimperman *et al*. 2002, Logina *et al.* 2006).

B. miyamotoi was found in 6 out of 2 038 (0.3%) ticks in mainland Finland. Previously, overall prevalences of around the same number have been reported from *I. ricinus* ticks in the archipelago of south-western Finland and in the Åland Islands (0.6% and 0.3%, respectively) (Wilhelmsson *et al.* 2013, Sormunen *et al*. 2016b). In Estonia, a significantly higher prevalence was reported in *I. persulcatus* than in *I. ricinus* (2.7% vs. 0.4%, respectively) among the collection of ~2 600 ticks (Geller *et al.* 2012). In our study, four ticks were *I. persulcatus* and two *I. ricinus*, but with such small numbers, no conclusions of the difference in the *B. miyamotoi* prevalence between the tick species can be made. The significance of *B. miyamotoi* as a new, emerging tick-borne pathogen in humans is still unclear, but the finding

that it is present in mainland Finland and in distinct geographical areas warrants more research.

In addition to tick-borne pathogens examined in our study (I), several other species with potential pathogenicity for humans, such as *R. helvetica* and *A. phagocytophilum,* have recently been detected in ticks in south-western Finland (Sormunen *et al*. 2016b, Sormunen *et al.* 2016c). Due to the restricted overlapping distributional area of *I. ricinus* and *I. persulcatus* ticks in Europe, relatively little research comparing the pathogen diversities in these two tick species has been done. To extend the knowledge of possible tick-borne diseases affecting Finnish patients, further research is needed. The world of tick-borne diseases has continuously expanded during the past couple of decades (Dantas-Torres *et al.* 2012, Parola *et al*. 2013). New species, strains, and genotypes of microorganisms with pathogenic potential are being listed as the methods used in molecular biology develop. When a causative agent of an infectious disease has traditionally been identified after the evoked suspicion due to clinical disease, many microorganisms nowadays are already known to be present in ticks before they are recognized as human pathogens. However, the ticks also harbor numerous microbes that do not cause disease in humans and even if they do, are not necessarily transmitted to humans by ticks (Telford and Wormser 2010). Thus, the expanding spectrum of potentially pathogenic tick-borne microorganisms requires thorough studies on tick and pathogen ecology in association with the studies on the epidemiology and pathophysiology of human disease in order to reveal the causality between the suspected pathogen and clinical disease.

6.3 Demographics of LB cases in Finland

A bimodal age-specific distribution of LB incidence has been reported in all epidemiological studies in Europe and the US and was also seen in both clinically diagnosed and microbiologically confirmed LB cases in our study (II). Plain explanations for these two incidence peaks seen in school-age children and older people cannot be found in the literature. Perhaps the peak in older age groups could be explained by more frequent exposure to tick bites due to certain outdoor activities such as gardening and berry picking, which might be more popular among older people in Finland. Furthermore, the aging immune system may predispose older people to develop disseminated LB, which is seen as an increased number of LB cases in NIDR and an increasing seroprevalence by age (II and III). However, one must keep in mind that observed seroprevalence in the adult population is also cumulative since the antibodies may persist for years after adequately treated LB infection (Hammers-Berggren *et al.* 1994).

Our data showing the female preponderance in clinically diagnosed LB cases (59.4%) is in accordance with previous epidemiological studies in Europe that have reported a similar female preponderance among EM patients (Asbrink *et al.* 1986, Stanek *et al.* 1987, Strle *et al.* 2002, Mehnert and Krause 2005, Bennet *et al.* 2007). Especially in adults between 50 to 79 years of age, the LB incidence was clearly higher in females in our study. Only among boys aged 5 to 14 and males over 80 years, the incidence of clinically diagnosed LB cases was slightly higher compared with females. Interestingly, of ~3 400 EM cases reported in south-eastern Sweden in 1997–2003, females represented 54.5%, and they attracted more tick bites compared with males, although they spent approximately 30% less time outdoors (Bennet *et al.* 2007).

In contrast, the gender-specific differences in LB incidence are not as notable when it comes to microbiologically confirmed LB cases. Across all age groups, females were slightly predominating, except in the older age groups (≥70 years), where males were clearly overrepresented. In an epidemiological study conducted in France, 52.0% of LB cases reported between 2004 and 2012 by general practitioners were females, whereas the proportion of males was clearly higher in hospitalized LB patients (57.8% vs. 42.2%) (Vandenesch *et al.* 2014). The weighed Bbsl IgG seroprevalence increased by age and was significantly higher among Finnish males than females (4.97% vs. 2.99%; p=0.005) (III). Thus, being a male and being elderly were significant risk factors for LB. However, there was a discrepancy between the gender-specific LB incidence (II) and Bbsl IgG seroprevalence (III). While the higher LB incidence has been reported in females than in males in our study (II) and in other incidence studies conducted in Europe (Strle 1999, Mehnert and Krause 2005, Bennet *et al.* 2007, Fülöp and Poggensee 2008), the seroprevalence has been higher among males (III) (Carlsson *et al.* 1998, Hjetland *et al.* 2014, Johansson *et al.* 2017). Females may notice EM more often or be more active to seek the health care services already in the EM phase of the infection. Immunological or biological explanations about why LB would disseminate more likely in males than in females are not known (Bennet *et al.* 2007).

6.4 Current situation and temporal changes in the geographical incidence of LB by hospital districts

To provide information on the current geographical distribution of LB incidence in Finland, the total annual number of LB cases (clinically diagnosed and microbiologically confirmed) by HDs in 2011–2015 were calculated. The average incidence in the past five years (rather than reporting 2015 alone) was used as “current

situation" because the year-to-year variation in annual LB incidences was relatively wide in some HDs. The geographical distribution of Bbsl IgG seroprevalence generally correlated well with the LB incidence as well as with the vector tick occurrence in Finland (I–III). The northern region showed a low seroprevalence while in southern and eastern regions, the seroprevalence was higher. In contrast, the seroprevalences of western and central regions differed from the LB incidence and tick distribution: in the western region, seroprevalence was low while the incidence and tick occurrence were high, and in the central region, seroprevalence was high while the LB incidence and tick occurrence were low. Different levels of awareness among physicians and citizens seeking for healthcare services or high variation in spatial distribution of Bbsl within regions might explain these differences. Moreover, there are no studies showing whether the geographical and temporal reporting practices are uniform within the country.

The incidence of microbiologically confirmed LB cases increased in almost all HDs in the southern half of Finland and in the Åland Islands during 1995–2015. Central-western parts of the country remain low incidence areas, with no significant change in incidence during the study period. Even during the short reporting period of clinically diagnosed LB cases (Avohilmo), the incidence increased significantly in 8 out of 21 HDs.

In addition to increased LB incidence, the increased geographical distribution of LB cases following the expanding range of ticks has also been reported, for example in the US (Mead 2015). Although the expansion of tick distribution northwards along the coast of Bothnian Bay compared with the 1950s survey (Öhman 1961) was observed (I), as clear evidence of the geographical expansion of LB incidence was not detected (II). However, in Vaasa, less than 10 microbiologically confirmed LB cases per 100 000 population were reported yearly until 2006, after which the incidence made a sharp increase, reaching the highest number of 52 annual LB cases per 100 000 population in 2013. Along the northern coast of Bothnian Bay (e.g., in Länsi-Pohja and Northern Ostrobothnia), ticks were clustered to the relatively small geographical area, leading to a few human-tick encounters, and consequently, to a small number of LB cases proportionated to the inhabitants of the whole HD region. Perhaps with a resolution more subtle than HDs, slighter differences in geographical LB incidence by time could have been observed.

6.5 Clinical manifestations of LB cases

LNB is the most common clinical manifestation of the disseminated LB in Europe and is for the most part caused by *B. garinii* (Stanek and Strle 2008). In contrast, in the US, LNB is rare, with an annual incidence of 0.07/100 000 population most

likely due to the absence of *B. garinii* (Dessau *et al.* 2015). In our study (II), the annual incidence of LNB varied from 0.4 to 2.4/100 000 population between 1996 and 2014. In Denmark, where LNB has been a mandatory clinical notifiable disease since 1991, an average annual incidence of antibody index-confirmed LNB (i.e., confirmed by demonstration of intrathecal antibody production) was 3.2/100 000 population in 2010–2012 (Dessau *et al.* 2015). Around the same incidences have been reported in Sweden in 1992–1993 (2.0/100 000 population) (Berglund *et al.* 1995) and in Germany (Würzburg region) in 1996–1997 (3.0/100 000 population) (Huppertz *et al.* 1999). Interestingly, LNB cases represented only 5–12 % of all annual LB cases in Hilmo (II), which seems quite low, considering that LNB is probably the most common manifestation of disseminated LB also in Finland. In the Danish study by Dessau *et al.*, the surveillance system based on manual notifications of LNB cases by physicians was more inaccurate, incomplete, and delayed in comparison with the automated, electronic laboratory-based surveillance. It is likely that the small number of LNB cases in Hilmo can similarly be explained by the reporting practices. The physicians notifying LNB cases probably use the ICD-10 code "A69.2" for LB instead of specifying the clinical manifestation as LNB with additional ICD-10 codes. Since the LNB cases were extracted from Hilmo by the combination of ICD-10 codes referring specifically to LNB, a big proportion of cases was probably missed. The same lack is suspected with LA cases (II).

6.6 Limitations in the reporting practices of LB cases

A few limitations related to these registers are acknowledged. First, the awareness of LB among health care professionals may vary geographically and temporally, causing differences in reporting practices and diagnostic activity. Especially in Avohilmo, the correct notifications of EM cases are dependent on the health care professionals (mainly general practitioners) reporting the cases. To avoid underestimation of the total number of LB cases in Finland due to known inaccuracies in reporting, the number of clinically diagnosed LB cases was adjusted for the correction factor. Second, Avohilmo does not cover visits in occupational and private health care. Therefore, the number of clinically diagnosed LB cases is undoubtedly somewhat underestimated, especially among the people of working age. However, NIDR includes notifications from all healthcare sectors (public, occupational, and private), and the patterns of LB incidence by time, age-groups, and genders are similar to those seen in Avohilmo. Third, the place of infection is not necessarily the one reported as the place of residence or healthcare service in NIDR or Avohilmo. Further, NIDR does not contain any clinical data. Thus, it cannot be known with certainty if a portion of the microbiological notifications are actually positive

due to previous infection instead of an acute LB infection, which could cause an overestimation of microbiologically confirmed LB cases. A register-linkage study on an individual level (based on each individuals' national identity code) is currently ongoing also to refine the incidence estimates provided by study II.

It can also be speculated whether there is overlap between Avohilmo and NIDR. This could be expected in cases where laboratory testing for LB is performed in primary health care, either with the aim of confirming atypical EM or to diagnose disseminated LB. According to the preliminary results of an ongoing register-linkage study, the overlap of Avohilmo and NIDR is small. For example, in the year 2014, 6.3% of the cases in Avohilmo were also included in NIDR, indicating that 93.7% of the LB cases in Avohilmo (diagnosed with the ICD-10 code "A69.2") were not microbiologically confirmed (Docent Jussi Sane, NIHW, unpublished observation). This shows that the vast majority of the Avohilmo cases were not included in NIDR, and thus, the possible double-counting of LB cases should not cause a major bias to the data.

6.7 MBL deficiency and LB

MBL deficiency proved to be a risk factor for disseminated LB in our study (IV). There is a great heterogeneity in the cut-offs for low MBL serum concentrations used in studies examining disease associations (Heitzeneder *et al.* 2012). In our study (IV), the cut-offs for diminished and deficient MBL serum concentrations were determined using a separate data of 201 serum samples, of which both the MBL serum concentration and the MBL pathway function were measured. The commercial ELISA-based assay (Wieslab Total Complement System Screen Classical, MBL, Alternative Pathways, Euro-Diagnostica) used to measure the MBL pathway function measures neoantigen (C5b-9), which is produced as a result of complement activation via the MBL pathway. In this assay, diminished and deficient MBL pathway functions (diminished <40% and deficient <10%, interpretation criteria according to the manufacturer's instructions) were associated with the MBL serum concentrations of 787 and 445 ng/ml, respectively. The association between diminished MBL pathway function and decreased MBL serum concentration was strong: there were only 5 of 201 (2.5%) samples in the analysis that diverted from this so that the MBL serum concentration was decreased (below 787 ng/ml) while the MBL pathway function was normal (>40%). In addition to MBL, collectin-11 is also known to bind to mannan, thereby activating the MBL pathway (Ma *et al.* 2013). These five samples could be of such nature where the MBL serum concentration is diminished, but collectin-11 concentration is sufficient to activate

the MBL pathway of the complement system. However, if sufficient MBL concentration would not have been crucial to activate the MBL pathway in this assay, more samples, whose MBL concentration is decreased but whose MBL pathway works normally due to sufficient serum level of an MBL pathway activator other than MBL (e.g., collectin-11), would have been expected. Even if collectin-11 was considered an activator of the MBL pathway in our study, it would hardly have affected the results. Ficolins (ficolin-1, -2, and -3) can also activate the complement system via the MBL pathway (Garred *et al.* 2009). However, only MBL and collectin-11 bind to mannan. Therefore, the functional assay (Wieslab Total Complement System Screen Classical, MBL, Alternative Pathways, Euro-Diagnostica) specifically detects MBL- (or collectin-11-) dependent activation of the MBL pathway (Seelen *et al.* 2005).

Out of 201 serum samples, 24 (11.9%) had an MBL concentration over 787 ng/ml but the MBL pathway function was decreased (<40%). In these samples, MBL concentration is probably sufficient to activate the MBL pathway normally but the stepwise proceeding of the pathway stops at some point after initiation and does not lead to the formation of MAC. Instead of MBL deficiency, these samples might have another complement component deficiency which explains the decreased function of the pathway. Thus, other complement deficiencies than MBL deficiency could predispose humans to disseminated LB as well and would be worthy of investigation in future.

According to our study (IV), MBL deficiency was more frequent among LB patients than among non-LB controls, suggesting that impaired function of the MBL pathway of the complement increases susceptibility to disseminated LB. The median MBL concentration was also significantly lower in LB patient samples compared with non-LB control samples. Furthermore, among LB patients who had IgG antibodies against the *B. afzelii* p18 antigen, MBL deficiency was even more frequent than among all LB patients. Previous studies have shown that IgG antibodies against the p18 (decorin binding protein A) antigen are seen more often in patients with the more severe course of the disease, and that the concentration of these antibodies is higher among LNB and LA patients than in patients with early EM (Oschmann *et al.* 1997, Heikkilä *et al.* 2002). Thus, we speculated that perhaps impaired MBL pathway function leads to decreased early-phase immune defense and, as an underlying factor, allows the spirochete dissemination, which is indicated by the presence of p18 IgG antibodies.

There was a declining trend in MBL serum concentration over age both in LB patients and non-LB controls. A similar trend was previously reported by Aittoniemi *et al*. in a study where the age-dependent variation of MBL serum concentrations in healthy Finnish children and adults were assessed (Aittoniemi *et al.*

1996). In non-LB controls representing our control population, no changes in the frequency of diminished MBL concentration by age-groups were seen, but the proportion of diminished MBL concentration was constantly ~25–30%. This aligns with previous studies that report the frequency of low MBL serum levels (using cut-off <500 ng/ml) affecting around one-fourth of the general population (Minchinton *et al.* 2002, Eisen *et al.* 2008).

MBL deficiency predisposes people to certain autoimmune diseases (e.g., systemic lupus erythematosus) (Davies *et al.* 1995), which might cause symptoms similar to LB. Instead of using data from blood donor samples as a control population, the non-LB controls were also selected from sera that were sent for testing of Bbsl serology in our laboratory. By this, we wanted to avoid the bias that low serum MBL concentration in the LB patient group would reflect a potential autoimmune predisposition in patients whose serum samples are subjected to Bbsl serology. This and the fact that the frequency of low MBL concentration in each age group of non-LB controls corresponded with the occurrence of low MBL concentration in the general population indicate that the low serum MBL level among LB patients is indeed associated with LB.

It is known based on previous studies that MBL serum concentrations vary remarkably among healthy individuals (Aittoniemi *et al.* 1996, Minchinton *et al.* 2002, Gröndahl-Yli-Hannuksela *et al.* 2013). Thus, straightforward conclusions of the association between MBL concentration and LB cannot be made on an individual basis. Like the limitations of study IV, the missing MBL pathway functional data and genotyping data are acknowledged. However, the association between MBL pathway function and determined MBL serum concentrations was significant. Thus, based on study IV, it seems plausible that patients with MBL deficiency are more susceptible to LB.

7 CONCLUSIONS

Although LB is a well-known tick-borne disease in Finland among healthcare personnel and the public, the disease burden has not been thoroughly evaluated previously. Meanwhile, the range of vector ticks has been reported to expand especially towards northern latitudes, but the distribution of the ticks has not been studied in Finland. The main aim of this study was to investigate the distribution of human-infesting *Ixodes* ticks and associated pathogens in Finland, the LB prevalence and incidence, and related risk factors among the general population. The key findings of the study were:

I. Both tick species transmitting pathogens to humans in Finland, *I. ricinus* and *I. persulcatus*, are widely abundant in the southern half of the country. During the past few decades, the range of the ticks has shifted clearly northwards, especially along the coast of Bothnian Bay. The overall prevalence of Bbsl in ticks is 16.9%, and the prevalence of TBEV 1.6%. *B. miyamotoi* can be found in ticks in mainland Finland.

II. LB incidence has increased significantly in Finland during the past two decades. The incidence of microbiologically confirmed LB cases representing disseminated infections has increased around fivefold from a few hundred cases in 1990s to around 1 900 cases in 2015. When clinically diagnosed LB cases (approximately 3 000 yearly on average) are considered, the estimated number of LB cases at the present is around 6000–7 000 yearly (incidence: ~125–130/100 000 population). Overall, slightly more LB cases are seen in females, and a bimodal age-specific distribution is seen in clinically diagnosed and microbiologically confirmed LB cases. Great variation occurs in the geographical distribution of LB cases within the country.

III. The prevalence of Bbsl IgG antibodies in the general Finnish adult population is 3.9%. The weighted seroprevalence is significantly higher in males than in females, and it increases with age. The highest seroprevalences are detected in southern, central, and eastern regions. No associations between chronic diseases and Bbsl seropositivity were detected.

IV. Diminished serum MBL concentrations indicating impaired MBL pathway function of the complement were observed more frequently in the LB patients than in the non-LB controls. Also, the age-adjusted median serum MBL concentrations were significantly lower in LB patients than in the non-LB controls. This suggest that a deficiency in the MBL pathway of the complement is a risk factor for developing antibody-positive LB.

These findings indicate that LB is an increasing public health concern in Finland, reflecting the situation in northern Europe in general. The results of the study are useful both for healthcare personnel and the public in informing the areas and seasons of the highest infection risk, as well as to guide preventive measures, especially to risk groups within the country.

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REFERENCES

Ackermann, R, Rehse-Küpper, B, Gollmer, E, and Schmidt, R (1988): Chronic neurologic manifestations of erythema migrans borreliosis. *Ann N Y Acad Sci* 539, 16-23.

Aguero-Rosenfeld, ME, Wang, G, Schwartz, I, and Wormser, GP (2005): Diagnosis of lyme borreliosis. *Clin Microbiol Rev* 18, 484-509.

Aittoniemi, J, Baer, M, Soppi, E, Vesikari, T, and Miettinen, A (1998): Mannan binding lectin deficiency and concomitant immunodefects. *Arch Dis Child* 78, 245-8.

Aittoniemi, J, Miettinen, A, Laippala, P, Isolauri, E, Viikari, J, Ruuska, T, and Soppi, E (1996): Age-dependent variation in the serum concentration of mannan-binding protein. *Acta Paediatr* 85, 906-9.

Akira, S, Uematsu, S, and Takeuchi, O (2006): Pathogen recognition and innate immunity. *Cell* 124, 783-801.

Alekseev, AN, Dubinina, HV, Van De Pol, I, and Schouls, LM (2001): Identification of *Ehrlichia* spp. and *Borrelia burgdorferi* in *Ixodes* ticks in the Baltic regions of Russia. *J Clin Microbiol* 39, 2237-42.

Amosov, ML, Lesniak, OM, Obraztsova, RG, Mel'nikov, VG, Bardina, TG, and Andreeva, EA (2000): Clinical characteristics of tick-borne encephalitis complicated by Lyme borreliosis. *Vopr Virusol* 45, 25-8.

Anderson, JF (1989): Epizootiology of *Borrelia* in *Ixodes* tick vectors and reservoir hosts. *Rev Infect Dis* 11 Suppl 6, S1451-9.

Anderson, JF, and Magnarelli, LA (2008): Biology of ticks. *Infect Dis Clin North Am* 22, 195-215.

Asbrink, E, Olsson, I, and Hovmark, A (1986): Erythema chronicum migrans Afzelius in Sweden. A study on 231 patients. *Zentralbl Bakteriol Mikrobiol Hyg A* 263, 229-36.

Aureli, S, Galuppi, R, Ostanello, F, Foley, JE, Bonoli, C, Rejmanek, D, Rocchi, G, Orlandi, E, and Tampieri, MP (2015): Abundance of questing ticks and molecular evidence for pathogens in ticks in three parks of Emilia-Romagna region of Northern Italy. *Ann Agric Environ Med* 22, 459-66.

Bacon, RM, Kugeler, KJ, Mead, PS (2008): Surveillance for Lyme disease--United States, 1992-2006. Atlanta, GA: Department of Health & Human Services, Centers for Disease Control and Prevention. *MMWR Surveill Summ* 57, 1-9.

Balashov, IS, Grigor'eva, LA, and Oliver, J (1998): Interspecies cross of ixodes ticks from the group *Ixodes ricinus-persulcatus*. *Dokl Akad Nauk* 361, 712-4.

Balcer, LJ, Winterkorn, JM, and Galetta, SL (1997): Neuro-ophthalmic manifestations of Lyme disease. *J Neuroophthalmol* 17, 108-21.

Barbour, AG (1984): Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med* 57, 521-5.

Barbour, AG, and Fish, D (1993): The biological and social phenomenon of Lyme disease. *Science* 260, 1610-6.

Barbour, AG, and Hayes, SF (1986): Biology of Borrelia species. *Microbiol Rev* 50, 381-400.

Baumann, D, Pusterla, N, Péter, O, Grimm, F, Fournier, PE, Schär, G, Bossart, W, Lutz, H, and Weber, R (2003): Fever after a tick bite: clinical manifestations and diagnosis of acute tick bite-associated infections in northeastern Switzerland. *Dtsch Med Wochenschr* 128, 1042-7.

Belongia, EA (2002): Epidemiology and impact of coinfections acquired from *Ixodes* ticks. *Vector Borne Zoonotic Dis* 2, 265-73.

Benach, JL, Fleit, HB, Habicht, GS, Coleman, JL, Bosler, EM, and Lane, BP (1984): Interactions of phagocytes with the Lyme disease spirochete: role of the Fc receptor. *J Infect Dis* 150, 497-507.

Benke, T, Gasse, T, Hittmair-Delazer, M, and Schmutzhard, E (1995): Lyme encephalopathy: long-term neuropsychological deficits years after acute neuroborreliosis. *Acta Neurol Scand* 91, 353-7.

Bennet, L, Halling, A, and Berglund, J (2006): Increased incidence of Lyme borreliosis in southern Sweden following mild winters and during warm, humid summers. *Eur J Clin Microbiol Infect Dis* 25, 426-32.

Bennet, L, Stjernberg, L, and Berglund, J (2007): Effect of gender on clinical and epidemiologic features of Lyme borreliosis. *Vector Borne Zoonotic Dis* 7, 34-41.

Bentas, W, Karch, H, and Huppertz, HI (2000): Lyme arthritis in children and adolescents:

outcome 12 months after initiation of antibiotic therapy. *J Rheumatol* 27, 2025-30.

Berende, A, Oosting, M, Kullberg, BJ, Netea, MG, and Joosten, LA (2010): Activation of innate host defense mechanisms by Borrelia. *Eur Cytokine Netw* 21, 7-18.

Berglund, J, Eitrem, R, Ornstein, K, Lindberg, A, Ringér, A, Elmrud, H, Carlsson, M, Runehagen, A, Svanborg, C, and Norrby, R (1995): An epidemiologic study of Lyme disease in southern Sweden. *N Engl J Med* 333, 1319-27.

Berglund, J, Stjernberg, L, Ornstein, K, Tykesson-Joelsson, K, and Walter, H (2002): 5-y Follow-up study of patients with neuroborreliosis. *Scand J Infect Dis* 34, 421-5.

Blanc, F, Jaulhac, B, Fleury, M, de Seze, J, de Martino, SJ, Remy, V, Blaison, G, Hansmann, Y, Christmann, D, and Tranchant, C (2007): Relevance of the antibody index to diagnose Lyme neuroborreliosis among seropositive patients. *Neurology* 69, 953-8.

Blanco, JR, and Oteo, JA (2002): Human granulocytic ehrlichiosis in Europe. *Clin Microbiol Infect* 8, 763-72.

Bloch, C, Friedl, A, Zucol, F, Widmer, A, and Khanna, N (2013): Fever and lymphadenopathy. Report of 4 cases of tularemia. *Internist (Berl)* 54, 491-7.

Boden, K, Lobenstein, S, Hermann, B, Margos, G, and Fingerle, V (2016): *Borrelia miyamotoi*-associated neuroborreliosis in immunocompromised person. *Emerg Infect Dis* 22, 1617-20.

Boone, I, Hassler, D, Nguyen, T, Splettstoesser, WD, Wagner-Wiening, C, and Pfaff, G (2015): Tularaemia in southwest Germany: Three cases of tick-borne transmission. *Ticks Tick Borne Dis* 6, 611-4.

Borchers, AT, Keen, CL, Huntley, AC, and Gershwin, ME (2015): Lyme disease: a rigorous review of diagnostic criteria and treatment. *J Autoimmun* 57, 82-115.

Bormane, A, Lucenko, I, Duks, A, Mavtchoutko, V, Ranka, R, Salmina, K, and Baumanis, V (2004): Vectors of tick-borne diseases and epidemiological situation in Latvia in 1993-2002. *Int J Med Microbiol* 293 Suppl 37, 36-47.

Bremell, D, and Dotevall, L (2014): Oral doxycycline for Lyme neuroborreliosis with symptoms of encephalitis, myelitis, vasculitis or intracranial hypertension. *Eur J Neurol* 21, 1162-7.

Brissette, CA, and Gaultney, RA (2014): That's my story, and I'm sticking to it--an update on *B. burgdorferi* adhesins. *Front Cell Infect Microbiol* 4.

Bugmyrin, SV, Bespyatova, LA, Korotkov, YS, Burenkova, LA, Belova, OA, Romanova, LIu, Kozlovskaya, LI, Karganova, GG, and Ieshko, EP (2013): Distribution of *Ixodes ricinus* and *I. persulcatus* ticks in southern Karelia (Russia). *Ticks Tick Borne Dis* 4, 57-62.

Burgdorfer, W, Barbour, AG, Hayes, SF, Benach, JL, Grunwaldt, E, and Davis, JP (1982): Lyme disease-a tick-borne spirochetosis? *Science* 216, 1317-9.

Carlsson, SA, Granlund, H, Nyman, D, and Wahlberg, P (1998): IgG seroprevalence of Lyme borreliosis in the population of the Aland Islands in Finland. *Scand J Infect Dis* 30, 501-3.

Cedzynski, M, Szemraj, J, Swierzko, AS, Bak-Romaniszyn, L, Banasik, M, Zeman, K, and Kilpatrick, DC (2004): Mannan-binding lectin insufficiency in children with recurrent infections of the respiratory system. *Clin Exp Immunol* 136, 304-11.

Cerar, T, Ogrinc, K, Cimperman, J, Lotric-Furlan, S, Strle, F, and Ruzić-Sabljić, E (2008): Validation of cultivation and PCR methods for diagnosis of Lyme neuroborreliosis. *J Clin Microbiol* 46, 3375-9.

Chen, SM, Dumler, JS, Bakken, JS, and Walker, DH (1994): Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol* 32, 589-95.

Chmielewska-Badora, J, Moniuszko, A, Żukiewicz-Sobczak, W, Zwoliński, J, Piątek, J, and Pancewicz, S (2012): Serological survey in persons occupationally exposed to tick-borne pathogens in cases of co-infections with *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Babesia microti*. *Ann Agric Environ Med* 19, 271-4.

Chowdri, HR, Gugliotta, JL, Berardi, VP, Goethert, HK, Molloy, PJ, Sterling, SL, and Telford, SR (2013): *Borrelia miyamotoi* infection presenting as human granulocytic anaplasmosis: a case report. *Ann Intern Med* 159, 21-7.

Christen, HJ, Hanefeld, F, Eiffert, H, and Thomssen, R (1993): Epidemiology and clinical manifestations of Lyme borreliosis in childhood. A prospective multicentre study

with special regard to neuroborreliosis. *Acta Paediatr* Suppl 386, 1-75.

Christova, I, and Komitova, R (2004): Clinical and epidemiological features of Lyme borreliosis in Bulgaria. *Wien Klin Wochenschr* 116, 42-6.

Christova, I, Schouls, L, van De Pol, I, Park, J, Panayotov, S, Lefterova, V, Kantardjiev, T, and Dumler, JS (2001): High prevalence of granulocytic *Ehrlichiae* and *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks from Bulgaria. *J Clin Microbiol* 39, 4172-4.

Cimperman, J, Maraspin, V, Lotric-Furlan, S, Ruzić-Sabljić, E, Avsic-Zupanc, T, Picken, RN, and Strle, F (1998): Concomitant infection with tick-borne encephalitis virus and *Borrelia burgdorferi* sensu lato in patients with acute meningitis or meningoencephalitis. *Infection* 26, 160-4.

Cimperman, J, Maraspin, V, Lotric-Furlan, S, Ruzić-Sabljić, E, Avsic-Zupanc, T, and Strle, F (2002): Double infection with tick borne encephalitis virus and *Borrelia burgdorferi* sensu lato. *Wien Klin Wochenschr* 114, 620-2.

Clark, KL, Leydet, B, and Hartman, S (2013): Lyme borreliosis in human patients in Florida and Georgia, USA. *Int J Med Sci* 10, 915-31.

Clark, KL, Leydet, BF, and Threlkeld, C (2014): Geographical and genospecies distribution of *Borrelia burgdorferi* sensu lato DNA detected in humans in the USA. *J Med Microbiol* 63, 674-84.

Coipan, EC, Jahfari, S, Fonville, M, Maassen, CB, van der Giessen, J, Takken, W, Takumi, K, and Sprong, H (2013): Spatiotemporal dynamics of emerging pathogens in questing *Ixodes ricinus*. *Front Cell Infect Microbiol* 3, 36.

Coipan, EC, Jahfari, S, Fonville, M, Oei, GA, Spanjaard, L, Takumi, K, Hovius, JW, and Sprong, H (2016): Imbalanced presence of *Borrelia burgdorferi* s.l. multilocus sequence types in clinical manifestations of Lyme borreliosis. *Infect Genet Evol* 42, 66-76.

Connolly, SE, and Benach, JL (2005): The versatile roles of antibodies in *Borrelia* infections. *Nat Rev Microbiol* 3, 411-20.

Corrain, R, Drigo, M, Fenati, M, Menandro, ML, Mondin, A, Pasotto, D, and Martini, M (2012): Study on ticks and tick-borne zoonoses in public parks in Italy. *Zoonoses Public Health* 59, 468-76.

Cutler, SJ, Ruzic-Sabljic, E, and Potkonjak, A (2017): Emerging borreliae - Expanding beyond Lyme borreliosis. *Mol Cell Probes* 31: 22-27.

Dahl, M, Tybjaerg-Hansen, A, Schnohr, P, and Nordestgaard, BG (2004): A population-based study of morbidity and mortality in mannose-binding lectin deficiency. *J Exp Med* 199, 1391-9.

Daikh, BE, Emerson, FE, Smith, RP, Lucas, FL, and McCarthy, CA (2013): Lyme arthritis: a comparison of presentation, synovial fluid analysis, and treatment course in children and adults. *Arthritis Care Res* 65, 1986-90.

Dantas-Torres, F, Chomel, BB, and Otranto, D (2012): Ticks and tick-borne diseases: a One Health perspective. *Trends Parasitol* 28, 437-46.

Das, BK, and Panda, AK (2015): MBL-2 polymorphisms (codon 54 and Y-221X) and low MBL levels are associated with susceptibility to multi organ dysfunction in P. falciparum malaria in Odisha, India. *Front Microbiol* 6, 778.

Davies, EJ, Snowden, N, Hillarby, MC, Carthy, D, Grennan, DM, Thomson, W, and Ollier, WE (1995): Mannose-binding protein gene polymorphism in systemic lupus erythematosus. *Arthritis Rheum* 38, 110-4.

de Taeye, SW, Kreuk, L, van Dam, AP, Hovius, JW, and Schuijt, TJ (2013): Complement evasion by Borrelia burgdorferi: it takes three to tango. *Trends Parasitol* 29, 119-28.

Dessau, RB, Espenhain, L, Mølbak, K, Krause, TG, and Voldstedlund, M (2015): Improving national surveillance of Lyme neuroborreliosis in Denmark through electronic reporting of specific antibody index testing from 2010 to 2012. *Euro Surveill* 20, 21184.

Dugat, T, Lagrée, AC, Maillard, R, Boulouis, HJ, and Haddad, N (2015): Opening the black box of *Anaplasma phagocytophilum* diversity: current situation and future perspectives. *Front Cell Infect Microbiol* 5, 61.

Dumler, JS (2012): The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. *FEMS Immunol Med Microbiol* 64, 13-20.

Duray, PH (1989): Histopathology of clinical phases of human Lyme disease. *Rheum Dis Clin North Am* 15, 691-710.

Dykhuizen, DE, Brisson, D, Sandigursky, S, Wormser, GP, Nowakowski, J, Nadelman, RB, and Schwartz, I (2008): The propensity of different *Borrelia burgdorferi* sensu stricto

genotypes to cause disseminated infections in humans. *Am J Trop Med Hyg* 78, 806-10.

Eikeland, R, Ljøstad, U, Mygland, A, Herlofson, K, and Løhaugen, GC (2012): European neuroborreliosis: neuropsychological findings 30 months post-treatment. *Eur J Neurol* 19, 480-7.

Eisen, DP, Dean, MM, Boermeester, MA, Fidler, KJ, Gordon, AC, Kronborg, G, Kun, JF, Lau, YL, Payeras, A, Valdimarsson, H, Brett, SJ, Ip, WK, Mila, J, Peters, MJ, Saevarsdottir, S, van Till, JW, Hinds, CJ, and McBryde, ES (2008): Low serum mannose-binding lectin level increases the risk of death due to pneumococcal infection. *Clin Infect Dis* 47, 510-6.

Eisen, DP, Dean, MM, Thomas, P, Marshall, P, Gerns, N, Heatley, S, Quinn, J, Minchinton, RM, and Lipman, J (2006): Low mannose-binding lectin function is associated with sepsis in adult patients. *FEMS Immunol Med Microbiol* 48, 274-82.

Eisen, DP, and Minchinton, RM (2003): Impact of mannose-binding lectin on susceptibility to infectious diseases. *Clin Infect Dis* 37, 1496-505.

Endo, Y, Matsushita, M, and Fujita, T (2011): The role of ficolins in the lectin pathway of innate immunity. *Int J Biochem Cell Biol* 43, 705-12.

Eriksson, P, Schröder, MT, Niiranen, K, Nevanlinna, A, Panelius, J, and Ranki, A (2013): The many faces of solitary and multiple erythema migrans. *Acta Derm Venereol* 93, 693-700.

Espí, A, Del Cerro, A, Somoano, A, García, VM, Prieto, J, Barandika, JF, García-Pérez, AL (2016): *Borrelia burgdorferi* sensu lato prevalence and diversity in ticks and small mammals in a Lyme borreliosis endemic Nature Reserve in North-Western Spain. Incidence in surrounding human populations. *Enferm Infecc Microbiol Clin.*

Estrada-Peña, A, and de la Fuente, J (2014): The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral Res* 108, 104-28.

Faulde, MK, and Robbins, RG (2008): Tick infestation risk and *Borrelia burgdorferi* s.l. infection-induced increase in host-finding efficacy of female *Ixodes ricinus* under natural conditions. *Exp Appl Acarol* 44, 137-45.

Fehr, JS, Bloemberg, GV, Ritter, C, Hombach, M, Lüscher, TF, Weber, R, and Keller, PM (2010): Septicemia caused by tick-borne bacterial pathogen *Candidatus* Neoehrlichia mikurensis. *Emerg Infect Dis* 16, 1127-9.

Fidler, KJ, Wilson, P, Davies, JC, Turner, MW, Peters, MJ, and Klein, NJ (2004): Increased incidence and severity of the systemic inflammatory response syndrome in patients deficient in mannose-binding lectin. *Intensive Care Med* 30, 1438-45.

Fish, AE, Pride, YB, and Pinto, DS (2008): Lyme carditis. *Infect Dis Clin North Am* 22, 275-88.

Fournier, PE, Allombert, C, Supputamongkol, Y, Caruso, G, Brouqui, P, and Raoult, D (2004): Aneruptive fever associated with antibodies to Rickettsia helvetica in Europe and Thailand. *J Clin Microbiol* 42, 816-8.

Fournier, PE, Grunnenberger, F, Jaulhac, B, Gastinger, G, and Raoult, D (2000): Evidence of *Rickettsia helvetica* infection in humans, eastern France. *Emerg Infect Dis* 6, 389-92.

Fraser, CM, Casjens, S, Huang, WM, Sutton, GG, Clayton, R, Lathigra, R, White, O, Ketchum, KA, Dodson, R, Hickey, EK, Gwinn, M, Dougherty, B, Tomb, JF, Fleischmann, RD, Richardson, D, Peterson, J, Kerlavage, AR, Quackenbush, J, Salzberg, S, Hanson, M, van Vugt, R, Palmer, N, Adams, MD, Gocayne, J, Weidman, J, Utterback, T, Watthey, L, McDonald, L, Artiach, P, Bowman, C, Garland, S, Fuji, C, Cotton, MD, Horst, K, Roberts, K, Hatch, B, Smith, HO, and Venter, JC (1997): Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 390, 580-6.

Fryland, L, Wilhelmsson, P, Lindgren, PE, Nyman, D, Ekerfelt, C, and Forsberg, P (2011): Low risk of developing *Borrelia burgdorferi* infection in the south-east of Sweden after being bitten by a *Borrelia burgdorferi*-infected tick. *Int J Infect Dis* 15, e174-81.

Fukunaga, M, Takahashi, Y, Tsuruta, Y, Matsushita, O, Ralph, D, McClelland, M, and Nakao, M (1995): Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. *Int J Syst Bacteriol* 45, 804-10.

Fülöp, B, and Poggensee, G (2008): Epidemiological situation of Lyme borreliosis in germany: surveillance data from six Eastern German States, 2002 to 2006. *Parasitol Res* 103 Suppl 1, S117-20.

Garred, P, Harboe, M, Oettinger, T, Koch, C, and Svejgaard, A (1994): Dual role of mannan-binding protein in infections: another case of heterosis? *Eur J Immunogenet* 21, 125-31.

Garred, P, Honoré, C, Ma, YJ, Munthe-Fog, L, and Hummelshøj, T (2009): MBL2, FCN1, FCN2 and FCN3-The genes behind the initiation of the lectin pathway of complement. *Mol Immunol* 46, 2737-44.

Garred, P, Larsen, F, Madsen, HO, and Koch, C (2003): Mannose-binding lectin deficiency--revisited. *Mol Immunol* 40, 73-84.

Garred, P, Madsen, HO, Balslev, U, Hofmann, B, Pedersen, C, Gerstoft, J, and Svejgaard, A (1997): Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. *Lancet* 349, 236-40.

Geller, J, Nazarova, L, Katargina, O, and Golovljova, I (2013): *Borrelia burgdorferi* sensu lato prevalence in tick populations in Estonia. *Parasit Vectors* 6, 202.

Geller, J, Nazarova, L, Katargina, O, Järvekülg, L, Fomenko, N, and Golovljova, I (2012): Detection and genetic characterization of relapsing fever spirochete *Borrelia miyamotoi* in Estonian ticks. *PLoS One* 7, e51914.

Glatz, M, Golestani, M, Kerl, H, and Müllegger, RR (2006): Clinical relevance of different IgG and IgM serum antibody responses to *Borrelia burgdorferi* after antibiotic therapy for erythema migrans: long-term follow-up study of 113 patients. *Arch Dermatol* 142, 862-8.

Gordon, AC, Waheed, U, Hansen, TK, Hitman, GA, Garrard, CS, Turner, MW, Klein, NJ, Brett, SJ, and Hinds, CJ (2006): Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcome. *Shock* 25, 88-93.

Grankvist, A, Sandelin, LL, Andersson, J, Fryland, L, Wilhelmsson, P, Lindgren, PE, Forsberg, P, and Wennerås, C (2015): Infections with *Candidatus* Neoehrlichia mikurensis and cytokine responses in 2 persons bitten by ticks, Sweden. *Emerg Infect Dis* 21, 1462-5.

Gray, JS (2008): *Ixodes ricinus* seasonal activity: Implications of global warming indicated by revisiting tick and weather data. *International Journal of Medical Microbiology* 298, Suppl 1, 19-24.

Gray, JS, Dautel, H, Estrada-Peña, A, Kahl, O, and Lindgren, E (2009): Effects of climate change on ticks and tick-borne diseases in europe. *Interdiscip Perspect Infect Dis* 2009, 593232.

Gröndahl-Yli-Hannuksela, K, Viander, M, Mertsola, J, and He, Q (2013): Increased risk of pertussis in adult patients with mannose-binding lectin deficiency. *APMIS* 121, 311-5.

Gugliotta, JL, Goethert, HK, Berardi, VP, and Telford, SR (2013): Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *N Engl J Med* 368, 240-5.

Gurycová, D, Tináková, K, Výrosteková, V, and Gacíková, E (2010): The incidence of tularemia in Slovakia in 1997-2008. *Epidemiol Mikrobiol Imunol* 59, 39-44.

Gustafson, R, Svenungsson, B, Forsgren, M, Gardulf, A, and Granström, M (1992): Two-year survey of the incidence of Lyme borreliosis and tick-borne encephalitis in a high-risk population in Sweden. *Eur J Clin Microbiol Infect Dis* 11, 894-900.

Haapasalo, K, Suomalainen, P, Sukura, A, Siikamaki, H, and Jokiranta, TS (2010): Fatal babesiosis in man, Finland, 2004. *Emerg Infect Dis* 16, 1116-8.

Haglund, M, and Günther, G (2003): Tick-borne encephalitis--pathogenesis, clinical course and long-term follow-up. *Vaccine* 21 Suppl 1, S11-8.

Hammers-Berggren, S, Lebech, AM, Karlsson, M, Svenungsson, B, Hansen, K, and Stiernstedt, G (1994): Serological follow-up after treatment of patients with erythema migrans and neuroborreliosis. *J Clin Microbiol* 32, 1519-25.

Hanincova, K, Mukherjee, P, Ogden, NH, Margos, G, Wormser, GP, Reed, KD, Meece, JK, Vandermause, MF, and Schwartz, I (2013): Multilocus sequence typing of *Borrelia burgdorferi* suggests existence of lineages with differential pathogenic properties in humans. *PLoS One* 8, e73066.

Hansen, K, and Lebech, AM (1992): The clinical and epidemiological profile of Lyme neuroborreliosis in Denmark 1985-1990. A prospective study of 187 patients with *Borrelia burgdorferi* specific intrathecal antibody production. *Brain* 115, 399-423.

Heikkilä, T, Seppälä, I, Saxen, H, Panelius, J, Yrjänäinen, H, and Lahdenne, P (2002): Species-specific serodiagnosis of Lyme arthritis and neuroborreliosis due to *Borrelia burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* by using decorin binding protein A. *J Clin Microbiol* 40, 453-60.

Heitzeneder, S, Seidel, M, Förster-Waldl, E, and Heitger, A (2012): Mannan-binding lectin deficiency - Good news, bad news, doesn't matter? *Clin Immunol* 143, 22-38.

Henningsson, AJ, Malmvall, BE, Ernerudh, J, Matussek, A, and Forsberg, P (2010):

Neuroborreliosis--an epidemiological, clinical and healthcare cost study from an endemic area in the south-east of Sweden. *Clin Microbiol Infect* 16, 1245-51.

Henningsson, AJ, Wilhelmsson, P, Gyllemark, P, Kozak, M, Matussek, A, Nyman, D, Ekerfelt, C, Lindgren, PE, and Forsberg, P (2015): Low risk of seroconversion or clinical disease in humans after a bite by an *Anaplasma phagocytophilum*-infected tick. *Ticks Tick Borne Dis* 6, 787-92.

Herwaldt, BL, Cacciò, S, Gherlinzoni, F, Aspöck, H, Slemenda, SB, Piccaluga, P, Martinelli, G, Edelhofer, R, Hollenstein, U, Poletti, G, Pampiglione, S, Löschenberger, K, Tura, S, and Pieniazek, NJ (2003): Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. *Emerg Infect Dis* 9, 942-8.

Hibberd, ML, Sumiya, M, Summerfield, JA, Booy, R, and Levin, M (1999): Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Meningococcal Research Group. *Lancet* 353, 1049-53.

Hillyard, PD (1996): *Ticks of north-west Europe*. Field Studies Council.

Hjetland, R, Nilsen, RM, Grude, N, and Ulvestad, E (2014): Seroprevalence of antibodies to *Borrelia burgdorferi* sensu lato in healthy adults from western Norway: risk factors and methodological aspects. *APMIS* 122, 1114-24.

Hoal-Van Helden, EG, Epstein, J, Victor, TC, Hon, D, Lewis, LA, Beyers, N, Zurakowski, D, Ezekowitz, AB, and Van Helden, PD (1999): Mannose-binding protein B allele confers protection against tuberculous meningitis. *Pediatr Res* 45, 459-64.

Hofhuis, A, van der Giessen, JW, Borgsteede, FH, Wielinga, PR, Notermans, DW, and van Pelt, W (2006): Lyme borreliosis in the Netherlands: strong increase in GP consultations and hospital admissions in past 10 years. *Euro Surveill* 11, E060622.2.

Holden, K, Hodzic, E, Feng, S, Freet, KJ, Lefebvre, RB, and Barthold, SW (2005): Coinfection with *Anaplasma phagocytophilum* alters *Borrelia burgdorferi* population distribution in C3H/HeN mice. *Infect Immun* 73, 3440-4.

Hornok, S, Meli, ML, Gönczi, E, Halász, E, Takács, N, Farkas, R, and Hofmann-Lehmann, R. (2014) Occurrence of ticks and prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in three types of urban biotopes: forests, parks and cemeteries. *Ticks Tick Borne Dis* 5, 785-9.

Hovius, JW, de Wever, B, Sohne, M, Brouwer, MC, Coumou, J, Wagemakers, A, Oei, A, Knol, H, Narasimhan, S, Hodiamont, CJ, Jahfari, S, Pals, ST, Horlings, HM, Fikrig, E, Sprong, H, and van Oers, MH (2013): A case of meningoencephalitis by the relapsing fever spirochaete *Borrelia miyamotoi* in Europe. *Lancet* 382, 658.

Hovius, JW, Levi, M, and Fikrig, E (2008): Salivating for knowledge: potential pharmacological agents in tick saliva. *PLoS Med* 5, e43.

Hubalek, Z., Stunzner, D., Halouzka, J., Sixl, W., Wendelin, I., Juricova, Z., and Sanogo, Y. O. 2003. Prevalence of borreliae in ixodid ticks from a floodplain forest ecosystem. *Wien Klin. Wochenschr* 115:121–124.

Hubálek, Z (2009): Epidemiology of lyme borreliosis. *Curr Probl Dermatol* 37, 31-50.

Huegli, D, Moret, J, Rais, O, Moosmann, Y, Erard, P, Malinverni, R, and Gern, L (2011): Prospective study on the incidence of infection by *Borrelia burgdorferi* sensu lato after a tick bite in a highly endemic area of Switzerland. *Ticks Tick Borne Dis* 2, 129-36.

Hunfeld, KP, Hildebrandt, A, and Gray, JS (2008): Babesiosis: recent insights into an ancient disease. *Int J Parasitol* 38, 1219-37.

Huppertz, HI, Böhme, M, Standaert, SM, Karch, H, and Plotkin, SA (1999): Incidence of Lyme borreliosis in the Würzburg region of Germany. *Eur J Clin Microbiol Infect Dis* 18, 697-703.

Huppertz, HI, Karch, H, Suschke, HJ, Döring, E, Ganser, G, Thon, A, and Bentas, W (1995): Lyme arthritis in European children and adolescents. The Pediatric Rheumatology Collaborative Group. *Arthritis Rheum* 38, 361-8.

Hvidsten, D, Stordal, F, Lager, M, Rognerud, B, Kristiansen, BE, Matussek, A, Gray, J, and Stuen, S (2015): *Borrelia burgdorferi* sensu lato-infected *Ixodes ricinus* collected from vegetation near the Arctic Circle. *Ticks Tick Borne Dis* 6, 768-73.

Hytönen, J, Hartiala, P, Oksi, J, and Viljanen, MK (2008): Borreliosis: recent research, diagnosis, and management. *Scand J Rheumatol* 37, 161-72.

Hytönen, J, Kortela, E, Waris, M, Puustinen, J, Salo, J, and Oksi, J (2014): CXCL13 and neopterin concentrations in cerebrospinal fluid

of patients with Lyme neuroborreliosis and other diseases that cause neuroinflammation. *J Neuroinflammation* 11, 103.

Härkänen, T, Karvanen, J, Tolonen, H, Lehtonen, R, Djerf, K, Juntunen, T, and Koskinen, S (2016): Systematic handling of missing data in complex study designs–experiences from the Health 2000 and 2011 Surveys. *Journal of Applied Statistics* 43, 2772-2790.

Häselbarth, K, Tenter, AM, Brade, V, Krieger, G, and Hunfeld, KP (2007): First case of human babesiosis in Germany - Clinical presentation and molecular characterisation of the pathogen. *Int J Med Microbiol* 297, 197-204.

Ip, WK, Takahashi, K, Ezekowitz, RA, and Stuart, LM (2009): Mannose-binding lectin and innate immunity. *Immunol Rev* 230, 9-21.

Ivacic, L, Reed, KD, Mitchell, PD, and Ghebranious, N (2007): A LightCycler TaqMan assay for detection of *Borrelia burgdorferi* sensu lato in clinical samples. *Diagn Microbiol Infect Dis* 57, 137-43.

Ivanova, A, Geller, J, Katargina, O, Värv, K, Lundkvist, Å, and Golovljova, I (2017): Detection of *Candidatus* Neoehrlichia mikurensis and *Ehrlichia muris* in Estonian ticks. *Ticks Tick Borne Dis* 8, 13-17.

Jado, I, Oteo, JA, Aldámiz, M, Gil, H, Escudero, R, Ibarra, V, Portu, J, Portillo, A, Lezaun, MJ, García-Amil, C, Rodríguez-Moreno, I, and Anda, P (2007): *Rickettsia monacensis* and human disease, Spain. *Emerg Infect Dis* 13, 1405-7.

Jaenson, TG, Jaenson, DG, Eisen, L, Petersson, E, and Lindgren, E (2012): Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. *Parasit Vectors* 5, 8.

Jaenson, TG, and Lindgren, E (2011): The range of *Ixodes ricinus* and the risk of contracting Lyme borreliosis will increase northwards when the vegetation period becomes longer. *Ticks Tick Borne Dis* 2, 44-9.

Johansson, M, Manfredsson, L, Wistedt, A, Serrander, L, and Tjernberg, I (2017): Significant variations in the seroprevalence of C6 ELISA antibodies in a highly endemic area for Lyme borreliosis: evaluation of age, sex and seasonal differences. *APMIS* 125, 476-481.

Johnston, YE, Duray, PH, Steere, AC, Kashgarian, M, Buza, J, Malawista, SE, and Askenase, PW (1985): Lyme arthritis. Spirochetes found in synovial microangiopathic lesions. *Am J Pathol* 118, 26-34.

Jongejan, F, and Uilenberg, G (2004): The global importance of ticks. *Parasitology* 129 Suppl, S3-14.

Jore, S, Viljugrein, H, Hofshagen, M, Brun-Hansen, H, Kristoffersen, AB, Nygård, K, Brun, E, Ottesen, P, Sævik, BK, and Ytrehus, B (2011): Multi-source analysis reveals latitudinal and altitudinal shifts in range of *Ixodes ricinus* at its northern distribution limit. *Parasit Vectors* 4, 84.

Jungnick, S, Margos, G, Rieger, M, Dzaferovic, E, Bent, SJ, Overzier, E, Silaghi, C, Walder, G, Wex, F, Koloczek, J, Sing, A, and Fingerle, V (2015): *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii*: Population structure and differential pathogenicity. *Int J Med Microbiol* 305, 673-81.

Junttila, J, Peltomaa, M, Soini, H, Marjamäki, M, and Viljanen, MK (1999): Prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* ticks in urban recreational areas of Helsinki. *J Clin Microbiol* 37, 1361-5.

Jääskeläinen, A, Tonteri, E, Pieninkeroinen, I, Sironen, T, Voutilainen, L, Kuusi, M, Vaheri, A, and Vapalahti, O (2016): Siberian subtype tick-borne encephalitis virus in *Ixodes ricinus* in a newly emerged focus, Finland. *Ticks Tick Borne Dis* 7, 216-23.

Jääskeläinen, AE, Sironen, T, Murueva, GB, Subbotina, N, Alekseev, AN, Castrén, J, Alitalo, I, Vaheri, A, and Vapalahti, O (2010): Tick-borne encephalitis virus in ticks in Finland, Russian Karelia and Buryatia. *J Gen Virol* 91, 2706-12.

Jääskeläinen, AE, Tikkakoski, T, Uzcátegui, NY, Alekseev, AN, Vaheri, A, and Vapalahti, O (2006): Siberian subtype tickborne encephalitis virus, Finland. *Emerg Infect Dis* 12, 1568-71.

Kahl, O, Gern, L, Eisen, L, and Lane, RS (2002): Ecological research on *Borrelia burgdorferi* sensu lato: terminology and some methodological pitfalls. *Lyme borreliosis: biology, epidemiology and control*, 29-46.

Kaiser, R (1999): The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994-98: a prospective study of 656 patients. *Brain* 122, 2067-78.

Kalish, RA, Kaplan, RF, Taylor, E, Jones-Woodward, L, Workman, K, and Steere, AC (2001a): Evaluation of study patients with Lyme disease, 10-20-year follow-up. *J Infect Dis* 183, 453-60.

Kalish, RA, McHugh, G, Granquist, J, Shea, B, Ruthazer, R, and Steere, AC (2001b):

Persistence of immunoglobulin M or immunoglobulin G antibody responses to *Borrelia burgdorferi* 10-20 years after active Lyme disease. *Clin Infect Dis* 33, 780-5.

Katargina, O, Geller, J, Ivanova, A, Värv, K, Tefanova, V, Vene, S, Lundkvist, Å, and Golovljova, I (2015): Detection and identification of *Rickettsia* species in *Ixodes* tick populations from Estonia. *Ticks Tick Borne Dis*.

Katargina, O, Russakova, S, Geller, J, Kondrusik, M, Zajkowska, J, Zygutiene, M, Bormane, A, Trofimova, J, and Golovljova, I (2013): Detection and characterization of tick-borne encephalitis virus in Baltic countries and eastern Poland. *PLoS One* 8, e61374.

Kawahara, M, Rikihisa, Y, Isogai, E, Takahashi, M, Misumi, H, Suto, C, Shibata, S, Zhang, C, and Tsuji, M (2004): Ultrastructure and phylogenetic analysis of '*Candidatus* Neoehrlichia mikurensis' in the family *Anaplasmataceae*, isolated from wild rats and found in Ixodes ovatus ticks. *Int J Syst Evol Microbiol* 54, 1837-43.

Kelesidis, T (2014): The cross-talk between spirochetal lipoproteins and immunity. *Front Immunol* 5, 310.

Kindstrand, E, Nilsson, BY, Hovmark, A, Pirskanen, R, and Asbrink, E (1997): Peripheral neuropathy in acrodermatitis chronica atrophicans - a late Borrelia manifestation. *Acta Neurol Scand* 95, 338-45.

Kjelland, V, Stuen, S, Skarpaas, T, and Slettan, A (2010): Prevalence and genotypes of *Borrelia burgdorferi* sensu lato infection in *Ixodes ricinus* ticks in southern Norway. *Scand J Infect Dis* 42, 579-85.

Kochi, SK, and Johnson, RC (1988): Role of immunoglobulin G in killing of *Borrelia burgdorferi* by the classical complement pathway. *Infect Immun* 56, 314-21.

Kovalchuka, L, Eglite, J, Lucenko, I, Zalite, M, Viksna, L, and Krumiņa, A (2012): Associations of HLA DR and DQ molecules with Lyme borreliosis in Latvian patients. *BMC Res Notes* 5, 438.

Kowalski, TJ, Berth, WL, Mathiason, MA, and Agger, WA (2011): Oral antibiotic treatment and long-term outcomes of Lyme facial nerve palsy. *Infection* 39, 239-45.

Kraiczy, P, Skerka, C, Brade, V, and Zipfel, PF (2001): Further characterization of complement regulator-acquiring surface proteins of *Borrelia burgdorferi*. *Infect Immun* 69, 7800-9.

Krause, PJ, Narasimhan, S, Wormser, GP, Barbour, AG, Platonov, AE, Brancato, J, Lepore, T, Dardick, K, Mamula, M, Rollend, L, Steeves, TK, Diuk-Wasser, M, Usmani-Brown, S, Williamson, P, Sarksyan, DS, Fikrig, E, Fish, D, and Group, TBD (2014): *Borrelia miyamotoi* sensu lato seroreactivity and seroprevalence in the northeastern United States. *Emerg Infect Dis* 20, 1183-90.

Krause, PJ, Narasimhan, S, Wormser, GP, Rollend, L, Fikrig, E, Lepore, T, Barbour, A, and Fish, D (2013): Human *Borrelia miyamotoi* infection in the United States. *N Engl J Med* 368, 291-3.

Krause, PJ, Schwab, J, Narasimhan, S, Brancato, J, Xu, G, and Rich, SM (2016): Hard tick relapsing fever caused by *Borrelia miyamotoi* in a child. *Pediatr Infect Dis J* 35, 1352-1354.

Kristoferitsch, W, Stanek, G, and Kunz, C (1986): Double infection with early summer meningoencephalitis virus and *Borrelia burgdorferi*. *Dtsch Med Wochenschr* 111, 861-4.

Kurtenbach, K, Hanincová, K, Tsao, JI, Margos, G, Fish, D, and Ogden, NH (2006): Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nat Rev Microbiol* 4, 660-9.

Kybicová, K, Baštová, K, and Malý, M, (2017): Detection of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in questing ticks *Ixodes ricinus* from the Czech Republic. *Ticks Tick Borne Dis,* 8, 483-487.

Lakos, A, Igari, Z, and Solymosi, N (2012): Recent lesson from a clinical and seroepidemiological survey: low positive predictive value of *Borrelia burgdorferi* antibody testing in a high risk population. *Adv Med Sci* 57, 356-63.

Lane, RS, Piesman, J, and Burgdorfer, W (1991): Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. *Annu Rev Entomol* 36, 587-609.

Lebech, AM (2002): Polymerase chain reaction in diagnosis of *Borrelia burgdorferi* infections and studies on taxonomic classification. *APMIS* Suppl, 1-40.

Lesnyak, O, Laikovskaya, E, Kufko, I, Bruinink, H, Baranova, N, and Rijpkema, S (1998): Clinical features of Lyme borreliosis in the middle Urals and distribution of *Borrelia burgdorferi* sensu lato species in local Ixodes persulcatus ticks. *Zentralbl Bakteriol* 288, 111-9.

Lindgren, E, and Gustafson, R (2001): Tick-borne encephalitis in Sweden and climate change. *Lancet* 358, 16-8.

Lindgren, E, Jaenson, TG, Menne, B, and World Health Organization (2006): Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures.

Lindgren, E, Tälleklint, L, and Polfeldt, T (2000): Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick *Ixodes ricinus*. *Environ Health Perspect* 108, 119-23.

Lindquist, L, and Vapalahti, O (2008): Tick-borne encephalitis. *Lancet* 371, 1861-71.

Lipscombe, RJ, Sumiya, M, Hill, AV, Lau, YL, Levinsky, RJ, Summerfield, JA, and Turner, MW (1992): High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. *Hum Mol Genet* 1, 709-15.

Ljøstad, U, and Mygland, A (2010): Remaining complaints 1 year after treatment for acute Lyme neuroborreliosis; frequency, pattern and risk factors. *Eur J Neurol* 17, 118-23.

Ljøstad, U, Skogvoll, E, Eikeland, R, Midgard, R, Skarpaas, T, Berg, A, and Mygland, A (2008): Oral doxycycline versus intravenous ceftriaxone for European Lyme neuroborreliosis: a multicentre, non-inferiority, double-blind, randomised trial. *Lancet Neurol* 7, 690-5.

Lledó, L, Gegúndez, MI, Giménez-Pardo, C, Álamo, R, Fernández-Soto, P, Nuncio, MS, and Saz, JV (2014): A seventeen-year epidemiological surveillance study of *Borrelia burgdorferi* infections in two provinces of northern Spain. *Int J Environ Res Public Health* 11, 1661-72.

Logar, M, Ruzić-Sabljić, E, Maraspin, V, Lotric-Furlan, S, Cimperman, J, Jurca, T, and Strle, F (2004): Comparison of erythema migrans caused by *Borrelia afzelii* and *Borrelia garinii*. *Infection* 32, 15-9.

Logina, I, Krumina, A, Karelis, G, Elsone, L, Viksna, L, Rozentale, B, and Donaghy, M (2006): Clinical features of double infection with tick-borne encephalitis and Lyme borreliosis transmitted by tick bite. *J Neurol Neurosurg Psychiatry* 77, 1350-3.

Ma, YJ, Skjoedt, MO, and Garred, P (2013): Collectin-11/MASP complex formation triggers activation of the lectin complement pathway--the fifth lectin pathway initiation complex. *J Innate Immun* 5, 242-50.

Madeddu, G, Mancini, F, Caddeo, A, Ciervo, A, Babudieri, S, Maida, I, Fiori, ML, Rezza, G, and Mura, MS (2012): *Rickettsia monacensis* as cause of Mediterranean spotted fever-like illness, Italy. *Emerg Infect Dis* 18, 702-4.

Madsen, HO, Garred, P, Kurtzhals, JA, Lamm, LU, Ryder, LP, Thiel, S, and Svejgaard, A (1994): A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics* 40, 37-44.

Maiwald, M, Oehme, R, March, O, Petney, TN, Kimmig, P, Naser, K, Zappe, HA, Hassler, D, and von Knebel Doeberitz, M (1998): Transmission risk of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks to humans in southwest Germany. *Epidemiol Infect* 121, 103-8.

Mather, TN, and Mather, ME (1990): Intrinsic competence of three ixodid ticks (Acari) as vectors of the Lyme disease spirochete. *J Med Entomol* 27, 646-50.

Maurin, M, and Gyuranecz, M (2016): Tularaemia: clinical aspects in Europe. *Lancet Infect Dis* 16, 113-24.

Maurin, M, Pelloux, I, Brion, JP, Del Banõ, JN, and Picard, A (2011): Human tularemia in France, 2006-2010. *Clin Infect Dis* 53, e133-41.

Mead, PS (2015): Epidemiology of Lyme disease. *Infect Dis Clin North Am* 29, 187-210.

Medlock, JM, Hansford, KM, Bormane, A, Derdakova, M, Estrada-Peña, A, George, JC, Golovljova, I, Jaenson, TG, Jensen, JK, Jensen, PM, Kazimirova, M, Oteo, JA, Papa, A, Pfister, K, Plantard, O, Randolph, SE, Rizzoli, A, Santos-Silva, MM, Sprong, H, Vial, L, Hendrickx, G, Zeller, H, and Van Bortel, W (2013): Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. *Parasit Vectors* 6, 1.

Medzhitov, R, and Janeway, C (2000): Innate immunity. *N Engl J Med* 343, 338-44.

Mehnert, WH, and Krause, G (2005): Surveillance of Lyme borreliosis in Germany, 2002 and 2003. *Euro Surveill* 10, 83-5.

Mejlon, HA, and Jaenson, TG (1993): Seasonal prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* in different vegetation types in Sweden. *Scand J Infect Dis* 25, 449-56.

Mehlhorn, H, Mehlhorn, T, Müller, M, Vogt, M, and Rissland, J. (2016) Tick survey for prevalent pathogens in peri-urban recreation sites in Saarland and Rhineland-Palatinate (Germany). *Parasitol Res* 115, 1167-72.

Meri, T, Amdahl, H, Lehtinen, MJ, Hyvärinen, S, McDowell, JV, Bhattacharjee, A, Meri, S,

Marconi, R, Goldman, A, and Jokiranta, TS (2013): Microbes bind complement inhibitor factor H via a common site. *PLoS Pathog* 9, e1003308.

Merle, NS, Church, SE, Fremeaux-Bacchi, V, and Roumenina, LT (2015a): Complement System Part I - Molecular Mechanisms of Activation and Regulation. *Front Immunol* 6, 262.

Merle, NS, Noe, R, Halbwachs-Mecarelli, L, Fremeaux-Bacchi, V, and Roumenina, LT (2015b): Complement System Part II: Role in Immunity. *Front Immunol* 6, 257.

Mickiene, A, Laiskonis, A, Günther, G, Vene, S, Lundkvist, A, and Lindquist, L (2002): Tickborne encephalitis in an area of high endemicity in Lithuania: disease severity and long-term prognosis. *Clin Infect Dis* 35, 650-8.

Milutinović, M, Masuzawa, T, Tomanović, S, Radulović, Z, Fukui, T, and Okamoto, Y (2008): *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Francisella tularensis* and their co-infections in host-seeking *Ixodes ricinus* ticks collected in Serbia. *Exp Appl Acarol* 45, 171-83.

Minchinton, RM, Dean, MM, Clark, TR, Heatley, S, and Mullighan, CG (2002): Analysis of the relationship between mannose-binding lectin (MBL) genotype, MBL levels and function in an Australian blood donor population. *Scand J Immunol* 56, 630-41.

Misonne, MC, Van Impe, G, and Hoet, PP. (1998): Genetic heterogeneity of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in Belgium. *J Clin Microbiol* 36, 3352-4.

Moniuszko-Malinowska, A, Swiecicka, I, Dunaj, J, Zajkowska, J, Czupryna, P, Zambrowski, G, Chmielewska-Badora, J, Żukiewicz-Sobczak, W, Swierzbinska, R, Rutkowski, K, Garkowski, A, and Pancewicz, S (2016): Infection with *Babesia microti* in humans with non-specific symptoms in North East Poland. *Infect Dis* 48, 537-43.

Moriarty, TJ, Norman, MU, Colarusso, P, Bankhead, T, Kubes, P, and Chaconas, G (2008): Real-time high resolution 3D imaging of the lyme disease spirochete adhering to and escaping from the vasculature of a living host. *PLoS Pathog* 4, e1000090.

Motaleb, MA, Corum, L, Bono, JL, Elias, AF, Rosa, P, Samuels, DS, and Charon, NW (2000): *Borrelia burgdorferi* periplasmic flagella have both skeletal and motility functions. *Proc Natl Acad Sci* 97, 10899-904.

Movila, A, Uspenskaia, I, Toderas, I, Melnic, V, and Conovalov, J (2006): Prevalence of *Borrelia burgdorferi* sensu lato and *Coxiella burnetti* in ticks collected in different biocenoses in the Republic of Moldova. *International Journal of Medical Microbiology* 296, 172-176.

Nadelman, RB, Nowakowski, J, Forseter, G, Goldberg, NS, Bittker, S, Cooper, D, Aguero-Rosenfeld, M, and Wormser, GP (1996): The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. *Am J Med* 100, 502-8.

Nahimana, I, Gern, L, Blanc, DS, Praz, G, Francioli, P, and Péter, O (2004): Risk of *Borrelia burgdorferi* infection in western Switzerland following a tick bite. *Eur J Clin Microbiol Infect Dis* 23, 603-8.

Nelson, CA, Saha, S, Kugeler, KJ, Delorey, MJ, Shankar, MB, Hinckley, AF, and Mead, PS (2015): Incidence of clinician-diagnosed Lyme disease, United States, 2005-2010. *Emerg Infect Dis* 21, 1625-31.

Nicholson, MC, and Mather, TN (1996): Methods for evaluating Lyme disease risks using geographic information systems and geospatial analysis. *J Med Entomol* 33, 711-20.

Nigrovic, LE, and Thompson, KM (2007): The Lyme vaccine: a cautionary tale. *Epidemiol Infect* 135, 1-8.

Nilsson, K (2009): Septicaemia with *Rickettsia helvetica* in a patient with acute febrile illness, rash and myasthenia. *J Infect* 58, 79-82.

Nilsson, K, Lindquist, O, Liu, AJ, Jaenson, TG, Friman, G, and Påhlson, C (1999a): *Rickettsia helvetica* in *Ixodes ricinus* ticks in Sweden. *J Clin Microbiol* 37, 400-3.

Nilsson, K, Lindquist, O, and Påhlson, C (1999b): Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. *Lancet* 354, 1169-73.

Nilsson, K, Wallménius, K, Hartwig, S, Norlander, T, and Påhlson, C (2013): Bell's palsy and sudden deafness associated with *Rickettsia* spp. infection in Sweden. A retrospective and prospective serological survey including PCR findings. *Eur J Neurol* 21, 206-14.

Nocton, JJ, Bloom, BJ, Rutledge, BJ, Persing, DH, Logigian, EL, Schmid, CH, and Steere, AC (1996): Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in cerebrospinal fluid in Lyme neuroborreliosis. *J Infect Dis* 174, 623-7.

Nunes, M, Parreira, R, Maia, C, Lopes, N, Fingerle, V, and Vieira, ML (2016): Molecular identification of *Borrelia* genus in questing hard ticks from Portugal: Phylogenetic characterization of two novel Relapsing Fever-like *Borrelia* sp. *Infect Genet Evol* 40, 266-74.

Nussenzweig, MC, Steinman, RM, Gutchinov, B, and Cohn, ZA (1980): Dendritic cells are accessory cells for the development of anti-trinitrophenyl cytotoxic T lymphocytes. *J Exp Med* 152, 1070-84.

Ogden, NH, Maarouf, A, Barker, IK, Bigras-Poulin, M, Lindsay, LR, Morshed, MG, O'callaghan, CJ, Ramay, F, Waltner-Toews, D, and Charron, DF (2006): Climate change and the potential for range expansion of the Lyme disease vector *Ixodes scapularis* in Canada. *Int J Parasitol* 36, 63-70.

Oksi, J, Marjamäki, M, Koski, K, Nikoskelainen, J, and Viljanen, MK (1995): Bilateral facial palsy and meningitis caused by borrelia double infection. *Lancet* 345, 1583-4.

Oksi, J, Marttila, H, Soini, H, Aho, H, Uksila, J, and Viljanen, MK (2001): Early dissemination of *Borrelia burgdorferi* without generalized symptoms in patients with erythema migrans. *APMIS* 109, 581-8.

Oksi, J, Nikoskelainen, J, Hiekkanen, H, Lauhio, A, Peltomaa, M, Pitkäranta, A, Nyman, D, Granlund, H, Carlsson, SA, Seppälä, I, Valtonen, V, and Viljanen, M (2007): Duration of antibiotic treatment in disseminated Lyme borreliosis: a double-blind, randomized, placebo-controlled, multicenter clinical study. *Eur J Clin Microbiol Infect Dis* 26, 571-81.

Oksi, J, Viljanen, MK, Kalimo, H, Peltonen, R, Marttía, R, Salomaa, P, Nikoskelainen, J, Budka, H, and Halonen, P (1993): Fatal encephalitis caused by concomitant infection with tick-borne encephalitis virus and *Borrelia burgdorferi*. *Clin Infect Dis* 16, 392-6.

Oosting, M, Buffen, K, van der Meer, JW, Netea, MG, and Joosten, LA (2014): Innate immunity networks during infection with *Borrelia burgdorferi*. *Crit Rev Microbiol*, 1-12.

Oosting, M, van de Veerdonk, FL, Kanneganti, TD, Sturm, P, Verschueren, I, Berende, A, van der Meer, JW, Kullberg, BJ, Netea, MG, and Joosten, LA (2011): *Borrelia* species induce inflammasome activation and IL-17 production through a caspase-1-dependent mechanism. *Eur J Immunol* 41, 172-81.

Oschmann, P, Dorndorf, W, Hornig, C, Schäfer, C, Wellensiek, HJ, and Pflughaupt, KW (1998): Stages and syndromes of neuroborreliosis. *J Neurol* 245, 262-72.

Oschmann, P, Wellensiek, HJ, Zhong, W, Dorndorf, W, and Pflughaupt, KW (1997): Relationship between the *Borrelia burgdorferi* specific immune response and different stages and syndromes in neuroborreliosis. *Infection* 25, 292-7.

Pangrácová, L, Derdáková, M, Pekárik, L, Hviščová, I, Víchová, B, Stanko, M, Hlavatá, H, and Peťko, B (2013): *Ixodes ricinus* abundance and its infection with the tick-borne pathogens in urban and suburban areas of Eastern Slovakia. *Parasit Vectors* 6, 238.

Parola, P, Paddock, CD, and Raoult, D (2005): Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev* 18, 719-56.

Parola, P, Paddock, CD, Socolovschi, C, Labruna, MB, Mediannikov, O, Kernif, T, Abdad, MY, Stenos, J, Bitam, I, Fournier, PE, and Raoult, D (2013): Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev* 26, 657-702.

Pausa, M, Pellis, V, Cinco, M, Giulianini, PG, Presani, G, Perticarari, S, Murgia, R, and Tedesco, F (2003): Serum-resistant strains of *Borrelia burgdorferi* evade complement-mediated killing by expressing a CD59-like complement inhibitory molecule. *J Immunol* 170, 3214-22.

Petersen JM, Schriefer ME (2005): Tularemia: emergence/re-emergence. *Vet Res* 36: 455-67.

Petrovec, M, Lotric Furlan, S, Zupanc, TA, Strle, F, Brouqui, P, Roux, V, and Dumler, JS (1997): Human disease in Europe caused by a granulocytic *Ehrlichia* species. *J Clin Microbiol* 35, 1556-9.

Petzke, MM, Brooks, A, Krupna, MA, Mordue, D, and Schwartz, I (2009): Recognition of *Borrelia burgdorferi*, the Lyme disease spirochete, by TLR7 and TLR9 induces a type I IFN response by human immune cells. *J Immunol* 183, 5279-92.

Pfäffle, M, Littwin, N, Muders, SV, and Petney, TN (2013): The ecology of tick-borne diseases. *Int J Parasitol* 43, 1059-77.

Pietikäinen, J, Meri, T, Blom, AM, and Meri, S (2010): Binding of the complement inhibitor C4b-binding protein to Lyme disease *Borreliae*. *Mol Immunol* 47, 1299-305.

Pisanu, B, Chapuis, JL, Dozières, A, Basset, F, Poux, V, and Vourc'h, G (2014): High

prevalence of *Borrelia burgdorferi* s.l. in the European red squirrel Sciurus vulgaris in France. *Ticks Tick Borne Dis* 5, 1-6.

Platonov, AE, Karan, LS, Kolyasnikova, NM, Makhneva, NA, Toporkova, MG, Maleev, VV, Fish, D, and Krause, PJ (2011): Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emerg Infect Dis* 17, 1816-23.

Porat, R, Poutsiaka, DD, Miller, LC, Granowitz, EV, and Dinarello, CA (1992): Interleukin-1 (IL-1) receptor blockade reduces endotoxin and *Borrelia burgdorferi* -stimulated IL-8 synthesis in human mononuclear cells. *FASEB J* 6, 2482-6.

Pritt, BS, Mead, PS, Johnson, DK, Neitzel, DF, Respicio-Kingry, LB, Davis, JP, Schiffman, E, Sloan, LM, Schriefer, ME, Replogle, AJ, Paskewitz, SM, Ray, JA, Bjork, J, Steward, CR, Deedon, A, Lee, X, Kingry, LC, Miller, TK, Feist, MA, Theel, ES, Patel, R, Irish, CL, and Petersen, JM (2016): Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. *Lancet Infect Dis* 16, 556-64.

Quarsten, H, Skarpaas, T, Fajs, L, Noraas, S, and Kjelland, V (2015): Tick-borne bacteria in *Ixodes ricinus* collected in southern Norway evaluated by a commercial kit and established real-time PCR protocols. *Ticks Tick Borne Dis* 6, 538-44.

Radolf, JD, Caimano, MJ, Stevenson, B, and Hu, LT (2012): Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev Microbiol* 10, 87-99.

Raileanu, C, Moutailler, S, Pavel, I, Porea, D, Mihalca, AD, Savuta, G, and Vayssier-Taussat, M (2017): *Borrelia* diversity and co-infection with other tick borne pathogens in ticks. *Front Cell Infect Microbiol* 7, 36.

Ramamoorthi, N, Narasimhan, S, Pal, U, Bao, F, Yang, XF, Fish, D, Anguita, J, Norgard, MV, Kantor, FS, Anderson, JF, Koski, RA, and Fikrig, E (2005): The Lyme disease agent exploits a tick protein to infect the mammalian host. *Nature* 436, 573-7.

Randolph, SE (2014): Ecology of non-nidicolous ticks. *Biology of ticks* 2, 3-38.

Ranka, R, Bormane, A, Salmina, K, and Baumanis, V (2004): Identification of three clinically relevant *Borrelia burgdorferi* sensu lato genospecies by PCR-restriction fragment length polymorphism analysis of 16S-23S ribosomal DNA spacer amplicons. *J Clin Microbiol* 42: 1444-9.

Rantala, A, Lajunen, T, Juvonen, R, Bloigu, A, Silvennoinen-Kassinen, S, Peitso, A, Saikku, P, Vainio, O, and Leinonen, M (2008): Mannose-binding lectin concentrations, MBL2 polymorphisms, and susceptibility to respiratory tract infections in young men. *J Infect Dis* 198, 1247-53.

Rar, VA, Livanova, NN, Panov, VV, Doroschenko, EK, Pukhovskaya, NM, Vysochina, NP, and Ivanov, LI (2010): Genetic diversity of *Anaplasma* and *Ehrlichia* in the Asian part of Russia. *Ticks Tick Borne Dis* 1, 57-65.

Rauter, C, and Hartung, T (2005): Prevalence of *Borrelia burgdorferi* sensu lato genospecies in *Ixodes ricinus* ticks in Europe: a metaanalysis. *Appl Environ Microbiol* 71, 7203-16.

Reis, C, Cote, M, Paul, RE, and Bonnet, S. (2011): Questing ticks in suburban forest are infected by at least six tick-borne pathogens. *Vector Borne Zoonotic Dis* 11, 907-16.

Ribeiro, JM, Mather, TN, Piesman, J, and Spielman, A (1987): Dissemination and salivary delivery of Lyme disease spirochetes in vector ticks (Acari: Ixodidae). *J Med Entomol* 24, 201-5.

Richter, D, Debski, A, Hubalek, Z, and Matuschka, FR (2012): Absence of Lyme disease spirochetes in larval *Ixodes ricinus* ticks. *Vector Borne Zoonotic Dis* 12, 21-7.

Rijpkema, S, Golubić, D, Molkenboer, M, Verbeek-De Kruif, N, and Schellekens, J. (1996): Identification of four genomic groups of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in a Lyme borreliosis endemic region of northern Croatia. *Exp Appl Acarol* 20, 23-30.

Rojko, T, Korva, M, Lotrič-Furlan, S, Strle, F, and Avšič-Županc, T (2016): Cluster of ulceroglandular tularemia cases in Slovenia. *Ticks Tick Borne Dis* 7, 1193-1197.

Rosen, ME, Hamer, SA, Gerhardt, RR, Jones, CJ, Muller, LI, Scott, MC, and Hickling, GJ (2012): *Borrelia burgdorferi* not detected in widespread *Ixodes scapularis* (Acari: Ixodidae) collected from white-tailed deer in Tennessee. *J Med Entomol* 49, 1473-80.

Rossow, H, Ollgren, J, Hytonen, J, Rissanen, H, Huitu, O, Henttonen, H, Kuusi, M, and Vapalahti, O (2015): Incidence and seroprevalence of tularaemia in Finland, 1995

to 2013: regional epidemics with cyclic pattern. *Euro Surveill* 20, 21209.

Rossow, H, Ollgren, J, Klemets, P, Pietarinen, I, Saikku, J, Pekkanen, E, Nikkari, S, Syrjälä, H, Kuusi, M, and Nuorti, JP (2014): Risk factors for pneumonic and ulceroglandular tularaemia in Finland: a population-based case-control study. *Epidemiol Infect* 142, 2207-16.

Roy, S, Knox, K, Segal, S, Griffiths, D, Moore, CE, Welsh, KI, Smarason, A, Day, NP, McPheat, WL, Crook, DW, Hill, AV, and Group, OPS (2002): MBL genotype and risk of invasive pneumococcal disease: a case-control study. *Lancet* 359, 1569-73.

Rudenko, N, Golovchenko, M, Grubhoffer, L, and Oliver, JH (2011): Updates on *Borrelia burgdorferi* sensu lato complex with respect to public health. *Ticks Tick Borne Dis* 2, 123-8.

Ruskamp, JM, Hoekstra, MO, Rovers, MM, Schilder, AG, and Sanders, EA (2006): Mannose-binding lectin and upper respiratory tract infections in children and adolescents: a review. *Arch Otolaryngol Head Neck Surg* 132, 482-6.

Salazar, JC, Duhnam-Ems, S, La Vake, C, Cruz, AR, Moore, MW, Caimano, MJ, Velez-Climent, L, Shupe, J, Krueger, W, and Radolf, JD (2009): Activation of human monocytes by live *Borrelia burgdorferi* generates TLR2-dependent and -independent responses which include induction of IFN-beta. *PLoS Pathog* 5, e1000444.

Salazar, JC, Pope, CD, Sellati, TJ, Feder, HM, Kiely, TG, Dardick, KR, Buckman, RL, Moore, MW, Caimano, MJ, Pope, JG, Krause, PJ, Radolf, JD, and Network, LD (2003): Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. *J Immunol* 171, 2660-70.

Sallenbach, S, Thiel, S, Aebi, C, Otth, M, Bigler, S, Jensenius, JC, Schlapbach, LJ, and Ammann, RA (2011): Serum concentrations of lectin-pathway components in healthy neonates, children and adults: mannan-binding lectin (MBL), M-, L-, and H-ficolin, and MBL-associated serine protease-2 (MASP-2). *Pediatr Allergy Immunol* 22, 424-30.

Santos, IK, Costa, CH, Krieger, H, Feitosa, MF, Zurakowski, D, Fardin, B, Gomes, RB, Weiner, DL, Harn, DA, Ezekowitz, RA, and Epstein, JE (2001): Mannan-binding lectin enhances susceptibility to visceral leishmaniasis. *Infect Immun* 69, 5212-5.

Sassera, D, Beninati, T, Bandi, C, Bouman, EA, Sacchi, L, Fabbi, M, and Lo, N (2006): '*Candidatus* Midichloria mitochondrii', an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. *Int J Syst Evol Microbiol* 56, 2535-2540.

Sastry, K, Herman, GA, Day, L, Deignan, E, Bruns, G, Morton, CC, and Ezekowitz, RA (1989): The human mannose-binding protein gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localization to chromosome 10. *J Exp Med* 170, 1175-89.

Sato, K, Takano, A, Konnai, S, Nakao, M, Ito, T, Koyama, K, Kaneko, M, Ohnishi, M, and Kawabata, H (2014): Human infections with *Borrelia miyamotoi*, Japan. *Emerg Infect Dis* 20, 1391-3.

Schorn, S, Pfister, K, Reulen, H, Mahling, M, and Silaghi, C (2011): Occurrence of *Babesia* spp., *Rickettsia* spp. and *Bartonella* spp. in *Ixodes ricinus* in Bavarian public parks, Germany. *Parasit Vectors* 4, 135.

Schotthoefer, AM, and Frost, HM (2015): Ecology and epidemiology of Lyme borreliosis. *Clin Lab Med* 35, 723-43.

Schouls, LM, Van De Pol, I, Rijpkema, SG, and Schot, CS (1999): Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. *J Clin Microbiol* 37, 2215-22.

Schuijt, TJ, Coumou, J, Narasimhan, S, Dai, J, Deponte, K, Wouters, D, Brouwer, M, Oei, A, Roelofs, JJ, van Dam, AP, van der Poll, T, Van't Veer, C, Hovius, JW, and Fikrig, E (2011): A tick mannose-binding lectin inhibitor interferes with the vertebrate complement cascade to enhance transmission of the lyme disease agent. *Cell Host Microbe* 10, 136-46.

Schwaiger, M, and Cassinotti, P (2003): Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA. *J Clin Virol* 27, 136-45.

Seelen, MA, Roos, A, Wieslander, J, Mollnes, TE, Sjöholm, AG, Wurzner, R, Loos, M, Tedesco, F, Sim, RB, Garred, P, Alexopoulos, E, Turner, MW, and Daha, MR (2005): Functional analysis of the classical, alternative, and MBL pathways of the complement system: standardization and validation of a simple ELISA. *J Immunol Methods* 296, 187-98.

Severinsson, K, Jaenson, TG, Pettersson, J, Falk, K, and Nilsson, K (2010): Detection and

prevalence of *Anaplasma phagocytophilum* and *Rickettsia helvetica* in *Ixodes ricinus* ticks in seven study areas in Sweden. *Parasit Vectors* 3, 66.

Silaghi, C, Beck, R, Oteo, JA, Pfeffer, M, and Sprong, H (2016): Neoehrlichiosis: an emerging tick-borne zoonosis caused by *Candidatus* Neoehrlichia mikurensis. *Exp Appl Acarol* 68, 279-97.

Smith, R, and Takkinen, J (2006): Lyme borreliosis: Europe-wide coordinated surveillance and action needed? *Euro Surveill* 11, E060622.

Sonenshine (1991): *Biology of Ticks*. Oxford University Press, New York, NY, US.

Sormunen, JJ, Klemola, T, Vesterinen, EJ, Vuorinen, I, Hytönen, J, Hänninen, J, Ruohomäki, K, Sääksjärvi, IE, Tonteri, E, and Penttinen, R (2016a): Assessing the abundance, seasonal questing activity, and *Borrelia* and tick-borne encephalitis virus (TBEV) prevalence of *Ixodes ricinus* ticks in a Lyme borreliosis endemic area in Southwest Finland. *Ticks Tick Borne Dis* 7, 208-15.

Sormunen, JJ, Penttinen, R, Klemola, T, Hänninen, J, Vuorinen, I, Laaksonen, M, Sääksjärvi, IE, Ruohomäki, K, and Vesterinen, EJ (2016b): Tick-borne bacterial pathogens in southwestern Finland. *Parasit Vectors* 9, 168.

Sormunen, JJ, Penttinen, R, Klemola, T, Vesterinen, EJ, and Hänninen, J (2016c): *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks in southwestern Finland. *Exp Appl Acarol* 70, 491-500.

Stafford, KC, Cartter, ML, Magnarelli, LA, Ertel, SH, and Mshar, PA (1998): Temporal correlations between tick abundance and prevalence of ticks infected with *Borrelia burgdorferi* and increasing incidence of Lyme disease. *J Clin Microbiol* 36, 1240-4.

Stanek, G, Fingerle, V, Hunfeld, KP, Jaulhac, B, Kaiser, R, Krause, A, Kristoferitsch, W, O'Connell, S, Ornstein, K, Strle, F, and Gray, J (2011): Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. *Clin Microbiol Infect* 17, 69-79.

Stanek, G, Flamm, H, Groh, V, Hirschl, A, Kristoferitsch, W, Neumann, R, Schmutzhard, E, and Wewalka, G (1987): Epidemiology of *Borrelia* infections in Austria. *Zentralbl Bakteriol Mikrobiol Hyg A* 263, 442-9.

Stanek, G, O'Connell, S, Cimmino, M, Aberer, E, Kristoferitsch, W, Granström, M, Guy, E, and Gray, J (1996): European Union Concerted Action on risk assessment in Lyme borreliosis: clinical case definitions for Lyme borreliosis. *Wien Klin Wochenschr* 108, 741-7.

Stanek, G, and Strle, F (2008): Lyme disease: European perspective. *Infect Dis Clin North Am* 22, 327-39, vii.

Stanek, G, and Strle, F (2009): Lyme borreliosis: a European perspective on diagnosis and clinical management. *Curr Opin Infect Dis* 22, 450-4.

Stanek, G, Wormser, GP, Gray, J, and Strle, F (2012): Lyme borreliosis. *Lancet* 379, 461-73.

Steere, AC, Bartenhagen, NH, Craft, JE, Hutchinson, GJ, Newman, JH, Rahn, DW, Sigal, LH, Spieler, PN, Stenn, KS, and Malawista, SE (1983a): The early clinical manifestations of Lyme disease. *Ann Intern Med* 99, 76-82.

Steere, AC, Duray, PH, and Butcher, EC (1988): Spirochetal antigens and lymphoid cell surface markers in Lyme synovitis. Comparison with rheumatoid synovium and tonsillar lymphoid tissue. *Arthritis Rheum* 31, 487-95.

Steere, AC, Klitz, W, Drouin, EE, Falk, BA, Kwok, WW, Nepom, GT, and Baxter-Lowe, LA (2006): Antibiotic-refractory Lyme arthritis is associated with HLA-DR molecules that bind a *Borrelia burgdorferi* peptide. *J Exp Med* 203, 961-71.

Steere, AC, Pachner, AR, and Malawista, SE (1983b): Neurologic abnormalities of Lyme disease: successful treatment with high-dose intravenous penicillin. *Ann Intern Med* 99, 767-72.

Steinman, RM (2012): Decisions about dendritic cells: past, present, and future. *Annu Rev Immunol* 30, 1-22.

Stensvold, CR, Al Marai, D, Andersen, LO, Krogfelt, KA, Jensen, JS, Larsen, KS, and Nielsen, HV (2015): *Babesia* spp. and other pathogens in ticks recovered from domestic dogs in Denmark. *Parasit Vectors* 8, 262.

Stojek, NM, and Dutkiewicz, J (2004): Studies on the occurrence of Gram-negative bacteria in ticks: *Ixodes ricinus* as a potential vector of Pasteurella. *Ann Agric Environ Med* 11, 319-22.

Strle, F (1999): Lyme borreliosis in Slovenia. *Zentralbl Bakteriol* 289, 643-52.

Strle, F (2004): Human granulocytic ehrlichiosis in Europe. *Int J Med Microbiol* 293 Suppl 37, 27-35.

Strle, F, Nelson, JA, Ruzic-Sabljic, E, Cimperman, J, Maraspin, V, Lotric-Furlan, S, Cheng, Y, Picken, MM, Trenholme, GM, and Picken, RN (1996): European Lyme borreliosis: 231 culture-confirmed cases involving patients with erythema migrans. *Clin Infect Dis* 23, 61-5.

Strle, F, and Stanek, G (2009): Clinical manifestations and diagnosis of Lyme borreliosis. *Curr Probl Dermatol* 37, 51-110.

Strle, F, Videcnik, J, Zorman, P, Cimperman, J, Lotric-Furlan, S, and Maraspin, V (2002): Clinical and epidemiological findings for patients with erythema migrans. Comparison of cohorts from the years 1993 and 2000. *Wien Klin Wochenschr* 114, 493-7.

Strle, K, Jones, KL, Drouin, EE, Li, X, and Steere, AC (2011): *Borrelia burgdorferi* RST1 (OspC type A) genotype is associated with greater inflammation and more severe Lyme disease. *Am J Pathol* 178, 2726-39.

Stuen, S, Granquist, EG, and Silaghi, C (2013): *Anaplasma phagocytophilum*--a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol* 3, 31.

Sudhindra, P, Wang, G, Schriefer, ME, McKenna, D, Zhuge, J, Krause, PJ, Marques, AR, and Wormser, GP (2016): Insights into *Borrelia miyamotoi* infection from an untreated case demonstrating relapsing fever, monocytosis and a positive C6 Lyme serology. *Diagn Microbiol Infect Dis* 86, 93-6.

Sumiya, M, Super, M, Tabona, P, Levinsky, RJ, Arai, T, Turner, MW, and Summerfield, JA (1991): Molecular basis of opsonic defect in immunodeficient children. *Lancet* 337, 1569-70.

Svendsen, CB, Krogfelt, KA, and Jensen, PM (2009): Detection of *Rickettsia* spp. in Danish ticks (Acari: *Ixodes ricinus*) using real-time PCR. *Scand J Infect Dis* 41, 70-2.

Svihrova, V, Hudeckova, H, Jesenak, M, Schwarzova, K, Kostanova, Z, and Ciznar, I (2011): Lyme borreliosis--analysis of the trends in Slovakia, 1999-2008. *Folia Microbiol* 56, 270-5.

Swanson, SJ, Neitzel, D, Reed, KD, and Belongia, EA (2006): Coinfections acquired from *Ixodes* ticks. *Clin Microbiol Rev* 19, 708-27.

Søborg, C, Madsen, HO, Andersen, AB, Lillebaek, T, Kok-Jensen, A, and Garred, P (2003): Mannose-binding lectin polymorphisms in clinical tuberculosis. *J Infect Dis* 188, 777-82.

Süss, J (2003): Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine* 21 Suppl 1, S19-35.

Süss, J, Schrader, C, Abel, U, Bormane, A, Duks, A, and Kalnina, V (2002): Characterization of tick-borne encephalitis (TBE) foci in Germany and Latvia (1997-2000). *Int J Med Microbiol* 291 Suppl 33, 34-42.

Takayama, K, Rothenberg, RJ, and Barbour, AG (1987): Absence of lipopolysaccharide in the Lyme disease spirochete, *Borrelia burgdorferi*. *Infect Immun* 55, 2311-3.

Taylor, ME, Brickell, PM, Craig, RK, and Summerfield, JA (1989): Structure and evolutionary origin of the gene encoding a human serum mannose-binding protein. *Biochem J* 262, 763-71.

Teillet, F, Dublet, B, Andrieu, JP, Gaboriaud, C, Arlaud, GJ, and Thielens, NM (2005): The two major oligomeric forms of human mannan-binding lectin: chemical characterization, carbohydrate-binding properties, and interaction with MBL-associated serine proteases. *J Immunol* 174, 2870-7.

Telford, SR, and Wormser, GP (2010): *Bartonella* spp. transmission by ticks not established. *Emerg Infect Dis* 16, 379-84.

Thiel, S, Vorup-Jensen, T, Stover, CM, Schwaeble, W, Laursen, SB, Poulsen, K, Willis, AC, Eggleton, P, Hansen, S, Holmskov, U, Reid, KB, and Jensenius, JC (1997): A second serine protease associated with mannan-binding lectin that activates complement. *Nature* 386, 506-10.

Thio, CL, Mosbruger, T, Astemborski, J, Greer, S, Kirk, GD, O'Brien, SJ, and Thomas, DL (2005): Mannose binding lectin genotypes influence recovery from hepatitis B virus infection. *J Virol* 79, 9192-6.

Thomas, HC, Foster, GR, Sumiya, M, McIntosh, D, Jack, DL, Turner, MW, and Summerfield, JA (1996): Mutation of gene of mannose-binding protein associated with chronic hepatitis B viral infection. *Lancet* 348, 1417-9.

Thomas, V, Anguita, J, Barthold, SW, and Fikrig, E (2001): Coinfection with *Borrelia burgdorferi* and the agent of human granulocytic ehrlichiosis alters murine immune responses, pathogen burden, and severity of Lyme arthritis. *Infect Immun* 69, 3359-71.

Tokarevich, NK, Tronin, AA, Blinova, OV, Buzinov, RV, Boltenkov, VP, Yurasova, ED, and Nurse, J (2011): The impact of climate change on the expansion of *Ixodes persulcatus* habitat and the incidence of tick-borne

encephalitis in the north of European Russia. *Glob Health Action* 4, 8448.

Tonteri, E, Jääskeläinen, AE, Tikkakoski, T, Voutilainen, L, Niemimaa, J, Henttonen, H, Vaheri, A, and Vapalahti, O (2011): Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008-2009. *Emerg Infect Dis* 17, 72-5.

Tonteri, E, Kipar, A, Voutilainen, L, Vene, S, Vaheri, A, Vapalahti, O, and Lundkvist, Å (2013): The three subtypes of tick-borne encephalitis virus induce encephalitis in a natural host, the bank vole (Myodes glareolus). *PLoS One* 8, e81214.

Tonteri, E, Kurkela, S, Timonen, S, Manni, T, Vuorinen, T, Kuusi, M, and Vapalahti, O (2015): Surveillance of endemic foci of tick-borne encephalitis in Finland 1995-2013: evidence of emergence of new foci. *Euro Surveill* 20.

Tälleklint, L, and Jaenson, TG (1994): Transmission of *Borrelia burgdorferi* s.l. from mammal reservoirs to the primary vector of Lyme borreliosis, *Ixodes ricinus* (Acari: *Ixodidae*), in Sweden. *J Med Entomol* 31, 880-6.

Tälleklint, L, and Jaenson, TG (1998): Increasing geographical distribution and density of *Ixodes ricinus* (Acari: *Ixodidae*) in central and northern Sweden. *J Med Entomol* 35, 521-6.

van Dam, AP, Oei, A, Jaspars, R, Fijen, C, Wilske, B, Spanjaard, L, and Dankert, J (1997): Complement-mediated serum sensitivity among spirochetes that cause Lyme disease. *Infect Immun* 65, 1228-36.

van der Linde, MR (1991): Lyme carditis: clinical characteristics of 105 cases. *Scand J Infect Dis* Suppl 77, 81-4.

van Duijvendijk, G, Coipan, C, Wagemakers, A, Fonville, M, Ersöz, J, Oei, A, Földvári, G, Hovius, J, Takken, W, and Sprong, H (2016): Larvae of *Ixodes ricinus* transmit *Borrelia afzelii* and *B. miyamotoi* to vertebrate hosts. *Parasit Vectors* 9, 97.

Vandenesch, A, Turbelin, C, Couturier, E, Arena, C, Jaulhac, B, Ferquel, E, Choumet, V, Saugeon, C, Coffinieres, E, Blanchon, T, Vaillant, V, and Hanslik, T (2014): Incidence and hospitalisation rates of Lyme borreliosis, France, 2004 to 2012. *Euro Surveill* 19.

Vannier, E, and Krause, PJ (2009): Update on babesiosis. *Interdiscip Perspect Infect Dis* 2009, 984568.

Viljanen, MK, and Punnonen, J (1989): The effect of storage of antigen-coated polystyrene microwells on the detection of antibodies against Borrelia burgdorferi by enzyme immunoassay (EIA). *J Immunol Methods* 124, 137-41.

von Loewenich, FD, Geissdörfer, W, Disqué, C, Matten, J, Schett, G, Sakka, SG, and Bogdan, C (2010): Detection of "*Candidatus* Neoehrlichia mikurensis" in two patients with severe febrile illnesses: evidence for a European sequence variant. *J Clin Microbiol* 48, 2630-5.

Wagemakers, A, Staarink, PJ, Sprong, H, and Hovius, JW (2015): *Borrelia miyamotoi*: a widespread tick-borne relapsing fever spirochete. *Trends Parasitol* 31; 260-69.

Wagner, E, and Frank, MM (2010): Therapeutic potential of complement modulation. *Nat Rev Drug Discov* 9, 43-56.

Wahlberg, P, Carlsson, SA, Granlund, H, Jansson, C, Lindén, M, Nyberg, C, and Nyman, D (2006): TBE in Åland Islands 1959-2005: Kumlinge disease. *Scand J Infect Dis* 38, 1057-62.

Wallis, R (2007): Interactions between mannose-binding lectin and MASPs during complement activation by the lectin pathway. *Immunobiology* 212, 289-99.

Wang, G, van Dam, AP, Schwartz, I, and Dankert, J (1999): Molecular typing of *Borrelia burgdorferi* sensu lato: taxonomic, epidemiological, and clinical implications. *Clin Microbiol Rev* 12, 633-53.

Weis, JJ, and Bockenstedt, L (2010): *Borrelia: molecular biology, host interaction and pathogenesis*. Horizon Scientific Press.

Welinder-Olsson, C, Kjellin, E, Vaht, K, Jacobsson, S, and Wennerås, C (2010): First case of human "*Candidatus* Neoehrlichia mikurensis" infection in a febrile patient with chronic lymphocytic leukemia. *J Clin Microbiol* 48, 1956-9.

Wilhelmsson, P, Fryland, L, Lindblom, P, Sjöwall, J, Ahlm, C, Berglund, J, Haglund, M, Henningsson, AJ, Nolskog, P, Nordberg, M, Nyberg, C, Ornstein, K, Nyman, D, Ekerfelt, C, Forsberg, P, and Lindgren, PE (2016): A prospective study on the incidence of *Borrelia burgdorferi* sensu lato infection after a tick bite in Sweden and on the Åland Islands, Finland (2008-2009). *Ticks Tick Borne Dis* 7, 71-9.

Wilhelmsson, P, Lindblom, P, Fryland, L, Ernerudh, J, Forsberg, P, and Lindgren, PE (2013): Prevalence, diversity, and load of

Borrelia species in ticks that have fed on humans in regions of Sweden and Åland Islands, Finland with different Lyme borreliosis incidences. *PLoS One* 8, e81433.

Wilking, H, and Stark, K (2014): Trends in surveillance data of human Lyme borreliosis from six federal states in eastern Germany, 2009-2012. *Ticks Tick Borne Dis* 5, 219-24.

Wodecka, B, Michalik, J, Lane, RS, Nowak-Chmura, M, and Wierzbicka, A (2016): Differential associations of *Borrelia* species with European badgers (*Meles meles*) and raccoon dogs (*Nyctereutes procyonoides*) in western Poland. *Ticks Tick Borne Dis* 7, 1010-6.

Woldehiwet, Z (2010): The natural history of *Anaplasma phagocytophilum*. *Vet Parasitol* 167, 108-22.

Wormser, GP, Brisson, D, Liveris, D, Hanincová, K, Sandigursky, S, Nowakowski, J, Nadelman, RB, Ludin, S, and Schwartz, I (2008): *Borrelia burgdorferi* genotype predicts the capacity for hematogenous dissemination during early Lyme disease. *J Infect Dis* 198, 1358-64.

Zagórski, Z, Biziorek, B, and Haszcz, D (2002): Ophthalmic manifestations in Lyme borreliosis. *Przegl Epidemiol* 56 Suppl 1, 85-90.

Zhao, X, Norris, SJ, and Liu, J (2014): Molecular architecture of the bacterial flagellar motor in cells. *Biochemistry* 53, 4323-33.

Öhman, C (1961): The geographical and topographical distribution of *I. ricinus* in Finland. *Acta Soc. Fauna Flora Fennica* 74, 1-38.

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